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The *Bulletin* was established to facilitate the dissemination and exchange of information among scientists working in the field of psychopharmacology. The emphasis is on rapid, informal reporting of work which has not been reported in the scientific literature. Publication in the *Bulletin* is not intended as a substitute for formal publication in journals. The content covers all phases of psychopharmacology, including descriptions of research programs and laboratories, progress reports on current research, descriptions of new methodologies and techniques, compilations of information about drugs, translations, summaries of conferences and symposia, lists of papers presented at scientific meetings, bibliographies, announcements of new publications, commentary and critique on specific problems and areas of research, and descriptions of the program and activities of the Psychopharmacology Service Center. Manuscripts appropriate for publication in the *Bulletin* are invited.

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*Research Conference on Drugs and Community Care: A Review and Analysis**

There has been increasing interest in recent years in the community care of psychotic patients, either following psychiatric hospitalizations or in lieu of such hospitalization. Both drugs and special clinical facilities, e.g., aftercare clinics or day-care facilities, have been used in the treatment of such patients. Although clinical opinions have, in general, favored combining drugs and special facilities, no well-designed, controlled research had been done on the subject until about 3 years ago, and, even at present, relatively little is being done.

This may be due in part to the reluctance of clinicians to accept some of the necessary conditions of controlled studies, such as the random assignment of patients to preselected types of treatment. It may have resulted, also, from the lack of methods for measuring change in psychiatric symptoms and in social adjustment of psychotic and post-psychotic outpatients. An Ad Hoc Committee on Followup Projects of the National Institute of Mental Health had made comments of this sort about the state of the field in 1955.

The Research Conference on Drugs and Community Care, sponsored by the Psychopharmacology Service Center of the National Institute of Mental Health, was organized partly in response to requests from research grantees of the Institute. In part, there was also a general feeling at the Center that enough interesting and productive research had been going on to warrant an exchange of ideas and of thoughts on the problems among investigators in the field. We were aware, for example (since these projects were being supported through research grants by the National Institute of Mental Health), that several well-controlled research projects, involving random assignment of patients to treatments under comparison, were being done or were in progress on such clinical problems as:

(1) the effectiveness of the combination of day-care and drugs in comparison with rehospitalization in the treatment of relapsed patients [Kris];

(2) the relapse rates of chronic schizophrenic outpatients on maintenance drug treatment in comparison with patients who were shifted gradually to placebo [Gross];

(3) the effectiveness of the combination of home care and drugs in contrast with that of hospitalization of acute schizophrenics [Pasamanick]; and

(4) a comparison of a potent tranquilizer, a mild tranquilizer, and a placebo in the treatment of outpatient psychotics [Engelhardt].

The criteria of treatment effectiveness in the above studies include relapse rate and several new measures of clinical condition and social adjustment.

Aside from its more general implications, the conference was expected to help highlight the clinical problems of investigators carrying out research of this type; the problems in collecting and analyzing data; difficulties in the selection of methods; and the practical implications of the important clinical results that have come out of these studies.

As background for the recommendations, which the authors will make on clinical practice and research design in this field, the purposes and structure of the conference will be described in the next section. That section is followed by summaries of the presentations of *research projects* and the conference sessions on *methodology*. The remaining three sections are devoted to the analysis of clinical results, the problems in research design, and recommendations on resolving some of the methodological issues in this area of clinical research.

STRUCTURE OF THE CONFERENCE

The conference was held in Washington, D.C., on September 19-20, 1960. Its primary objectives were:

(1) to permit investigators, carrying out research on drugs and community care with support of the National Institute of Mental Health, to discuss problems and to exchange ideas and experiences relevant to the solution of these problems;

(2) to provide the Center, its consultants, and the participating research investigators with a perspective of the research being supported;

(3) to determine what has been learned from these studies on drugs in the community care of psychotics,

* Prepared by Martin M. Katz, Research Psychologist, Psychopharmacology Service Center, and by Jonathan O. Cole, Chief, Psychopharmacology Service Center, Research Grants and Fellowships Branch, National Institute of Mental Health. This report is based on a research conference sponsored by the Psychopharmacology Service Center.

and

(4) to assess the technical aspects of such research, e.g., the establishment of criteria and the development of methods for classifying patients and for evaluating change and adjustment.

In addition to NIMH grantees conducting research on drug therapy in the community, the participants included investigators with related interests and experience and several who were just entering this field.

The conference was organized by Martin M. Katz. The participants were Dean J. Clyde, Biometrics Laboratory, George Washington University, Washington, D.C.; Jonathan O. Cole, NIMH; Joel J. Elkes, NIMH; David M. Engelhardt, State University of New York, Brooklyn; Leon Epstein, Langley Porter Clinic, San Francisco; Seymour Fisher, Syracuse University, New York; Robert Freedman, State University of New York, Brooklyn; Sol L. Garfield, Nebraska Psychiatric Institute, Omaha; Goldine Gleser, University of Cincinnati, Ohio; Bernard Glueck, Institute of Living, Hartford, Connecticut; Milton Greenblatt, Massachusetts Mental Health Center, Boston; Martin Gross, Springfield State Hospital, Sykesville, Maryland. Also participating were Leo E. Hollister, Veterans Administration Hospital, Palo Alto, California; Else B. Kris, Manhattan Aftercare Clinic, New York City; Jordan Lawrence, Springfield (Maryland) State Hospital; Mark Lefton, Columbus Psychiatric Institute, Ohio; Samuel B. Lyerly, Human Ecology Fund, New York City; David Mann, State University of New York, Brooklyn; Richard D. Morgan, Department of Mental Hygiene, Sacramento, California; Benjamin Pasamanick, Columbus Psychiatric Institute; Leonard Pearlin, NIMH; Seymour Perlin, Montifiori Hospital, New York; George A. Ulett, Washington University Medical School, St. Louis; and Joseph Zubin, Columbia University, New York.

The first half of the program was devoted to reports on six research projects, each of which was related to some aspect of drugs and community care. The papers reporting the projects emphasized aims, research design, methodological and operational problems, and plans for future work.

The second half of the conference was devoted to theoretical and practical problems of methodology. New methods and methodological problems mentioned briefly in the research reports were presented in greater detail and discussed more fully during the second half of the meeting.

CONFERENCE PROCEEDINGS

A. Summaries of the Six Research Projects

(1) Home Versus Hospital Care for Schizophrenics [Pasamanick]. This study is designed to test the hypothesis that acute schizophrenic patients can be treated adequately at home when psychiatric and public health nursing care is combined with appropriate drug therapy. All first-admission schizophrenic patients referred to the Columbus State Hospital are to be considered for the study. Those who are suicidal, homicidal, or so violently disturbed that it would be impossible to keep them at home will be admitted to the hospital but excluded from the study. The remaining patients will then be randomly assigned to one of the following three treatment groups: (*a*) a group treated at home with drugs, plus frequent visits by a public health nurse; (*b*) a group treated at home with placebo plus frequent visits by a public health nurse; and (*c*) a hospitalized group treated with the usual methods of the hospital. The three groups are to be evaluated before treatment, after 1 year, and after 2 years by psychiatric and psychological examinations, and by reports and ratings from nurses and social workers (including interview data from patients and their families). Quality of housing and general home environment of all three groups will also be rated.

A public health nurse, working in consultation with the psychiatrist and social worker, will visit frequently each home-care patient to give nursing guidance and counsel to the patient and his family. Patients in the home-care groups will be hospitalized when, on the basis of previously established criteria, hospitalization is deemed mandatory by the treatment teams.

The hospitalized group and the home-treatment groups are to be compared for total length of hospitalization, psychiatric condition, and family reaction and receptivity. The two home-care groups—one on placebo, one on drug—will be compared for a rate of hospital admission.

Discussion of this study was devoted to such matters as control of medication in the home-care groups, degree of comparability of the hospitalized and home-treatment groups, and problems related to hospitalization of patients assigned to home treatment. Later in the conference, Lefton, also from the Columbus Psychiatric Institute, presented a detailed discussion of the interview schedules which will be used in the study.

(2) A Study of Ataractics in Outpatient Schizophrenia [Engelhardt, Freedman]. This project was described as a longitudinal study of the effects of tranquilizers on the community adaptation of schizophrenic outpatients. Questions of interest in the study are whether continuation of medication, after an initial favorable effect, will prevent relapse, whether further improvement is noted when medication is continued, and, when there has been no initial benefit, whether improvement will occur after prolonged administration of drug. Changes in symptomatology and in social behavior associated with drug therapy are assessed after a brief time and after sustained administration of drug. The following criteria of

outpatient adaptation are being used: maintenance of outpatient status (i.e., avoidance of hospitalization), reduction in psychopathology, freedom from decrements in psychological functioning, reduction in social dysfunctioning, concordance between social adaptation and psychopathological changes, and stability of clinical course during a prolonged period (up to 24 months) of continuous treatment with drug or placebo.

Baseline psychiatric and psychological data are obtained during the first week. The patient is then placed on promazine, chlorpromazine, or placebo. Dosage levels are flexible and medication is administered double-blind. Supportive psychotherapy is given, but the emphasis is on medication. Patients are seen in the clinic at frequent intervals. Detailed re-evaluations are conducted after 3 months and at the end of 1 year.

Preliminary findings reported by Engelhardt show that the rehospitalization rate is significantly lower in the drug groups than in the placebo group. Also, the number of patients showing clinical improvement at 6 months is much higher in the drug groups than in patients on placebo, as is the rate of improvement of a group of patients who demonstrate severe thought-disturbance on initial evaluation. Further, the amount of social dysfunctioning as reported by a relative has been found to be greater in the placebo group than in the patients on chlorpromazine.

A detailed report on the methods of collection and analysis of the data on social behavior was presented later in the meeting by Mann and Freedman.

Freedman also discussed the problem of patient drop-out, which has been one of the major difficulties thus far. Attempts to distinguish clear-cut differences in personality or psychopathological features in the patients who drop out have not been successful, although the speculation is that there may be a complex interaction between the patient's expectation concerning treatment and what he actually experiences in the clinic.

Discussion of this project centered upon the specific kinds of information obtained from the patient's relatives, the handling of such data, problems of following up patients who drop out of the study, possible reasons for dropping out, side effects (which have not been a problem), the ethics of using placebos, and the possible relation between degree of social dysfunction and level of drug dosage.

(3) Drug Therapy in a Daycare Facility for Relapse Control [Kris]. The aim of this project is to evaluate hospital day-care plus drug therapy as a means of controlling relapse and preventing rehospitalization of formerly hospitalized psychotics, primarily schizophrenics. Acutely disturbed (relapsed) patients who would ordinarily be rehospitalized are randomly assigned to one of two treatments: (*a*) rehospitalization and usual hospital care, or (*b*) drug therapy in a special day-hospital affiliated with the Research Unit of the Manhattan Aftercare Clinic. At the time of assignment to a treatment group, the intensity of each patient's psychopathology is assessed by use of the Wittenborn Psychiatric Rating Scales. Patients assigned to the day-hospital are immediately started on intensive pharmacotherapy, with drug dosage individualized according to patients' needs.

Length of time between onset of relapse and subsequent remission in the two treatment groups, and community adaptation following remission, are being compared. After patients are released from either the hospital or the day-hospital, the investigators will study the patients further to determine whether remission achieved in the day-hospital is temporary or lasting.

Community adaptation is being measured by a set of scales developed by Katz, who described them more fully later in the conference.

Kris reported that the most remarkable finding thus far has been the rapid achievement of remission in the day-hospital patients, who return to their jobs far sooner than patients who were rehospitalized. She also noted that treatment in the day-hospital seems to promote better community adjustment and that patients seen in the day-hospital seem to have learned that they can discuss recurrences of symptoms without fear of being rehospitalized.

Questions about the details of handling patients at the day-hospital were discussed, along with comments about the liaison between the day-hospital and employment agencies or vocational rehabilitation centers, possibilities of using the center as a training facility for physicians and psychiatrists in private practice, criteria for admission to the study, and the staffing and physical layout of the day-hospital.

(4) Termination of Treatment with Ataractic Drugs [Gross]. This project, begun in 1958, investigated the importance of continuing chronic psychotic patients on medication after their release from the hospital. All patients in the study were first stabilized on one of six drugs and then randomly assigned to either (*a*) a control group which was continued on active medication, or (*b*) an experimental group which was gradually transferred from drug to placebo under double-blind conditions. Patients who relapsed were removed from the study and placed on medication if they had been receiving placebo or were rehospitalized. The criterion for relapse was the clinical judgment of the treating psychiatrist. A rating scale, developed to permit objective determination of the psychiatric condition and social adjustment of the patients, was described by Lawrence during the second half of the conference.

During the preliminary phase of the study, and at intervals thereafter, patients were evaluated by psy-

chological tests, and their families or the people with whom they were living were interviewed by a social worker. Frequency of relapse while on active medication was compared with that which occurred while patients were on placebo.

Summarizing some of the findings from the study, Gross reported that relapse occurred significantly more frequently during the withdrawal or placebo period, the relapse rate being approximately three times as high during the placebo period as during the period on medication. He also noted that three-fourths of the patients who relapsed while on placebo did not require rehospitalization but were able to regain stability after medication was resumed.

Among the problems of methodology and design which Gross enumerated were the difficulties experienced in dealing with six different drugs rather than with a single drug. He noted also that the generalizability of the findings was limited in that patients in the project—chronic psychotics who were free from alcoholism and organic brain damage and who were able to attend the clinic regularly—could not be considered representative of the general outpatient clinic population.

(5) Drugs and Social Therapy in Chronic Schizophrenia [Greenblatt]. This study was initiated to determine (*a*) how much the clinical change associated with drug treatment is due to the drug per se and how much to other causes, and (*b*) whether there are significant social and environmental differences between hospitals which may account for the differences in the effectiveness of drugs in one setting and another.

Sixty chronic schizophrenics were transferred from a State hospital to an intensive treatment center, the Massachusetts Mental Health Center, where 33 received drugs in addition to other therapy and 27 were not given drugs. Comparison groups were composed of 55 patients remaining at the State hospital, of whom 25 were treated with drugs and 20 were not. In neither setting were the patients assigned to "research wards." The criteria with which patients in the four groups were compared were clinical improvement and discharge rate.

Findings reported by Greenblatt showed only slight differences between the State hospital groups and the MMHC groups after 6 months. At 18 months, however, there were differences which suggested the possibility of a beneficial carry-over of milieu effects in patients who had originally been transferred to the MMHC.

In commenting on discharge rate, Greenblatt indicated that the State hospital's criteria for discharge are much more stringent than those of the MMHC. He also noted that discharge rate was affected by the presence of a family or a transitional facility to which the patients could be released. Among other special problems which he discussed were the difficulties of incorporating chronic schizophrenic patients into MMHC treatment routine without changing the environment of the Center, the reasons for having decided not to attempt double-blind administration of drug and placebo, and the possible significance of any manifestations of "transfer trauma" in patients moved from one setting to another.

(6) The Effect of Ataractic Drugs on Hospital Release Rates [Morgan and Epstein]. This project is one aspect of a much broader study of population movement in the California State Mental Hospitals. Morgan's paper was devoted to the overall study, and Epstein's to the parts of it which are specifically concerned with drug therapy.

Morgan first briefly explained the system of cohort followup analysis which is being used, noting that it is essentially the application of techniques of analyzing individual followup to a group of patients who have one or more characteristics in common—e.g., age, year of admission, sex, diagnosis, etc. Having set July 1948 as the beginning point for the collection of data, the California State Department of Mental Hygiene is now systematically coding and punching IBM cards with detailed information on all first-admission patients in the State's 11-hospital system. The records are not restricted to the period of first admission, but cover residence during subsequent readmissions in the same hospital or in a different one with the State system. A wealth of information is collected for each patient: vital statistics, diagnosis and details of treatment, and data covering current hospital entry, previous hospitalizations, leaves, etc. Thus, a patient's complete record of hospitalization can be examined in great detail at any point during the followup period, and cohorts can be constructed on the basis of any combination of a large number of descriptive characteristics.

Morgan observed that this technique permits analysis of the frequency of the likelihood of occurrence of changes in status during any specified interval in the followup. The status of a given cohort of patients can be examined for any period of time. Data being collected in this study will prove valuable in studies of current administrative policies and in investigations of the effectiveness of new or expanded programs. This system, Morgan said, is also valuable in providing retrospective control data which may be used in lieu of a control group in evaluations of specific programs.

Following Morgan's report, Epstein discussed a preliminary set of analyses of these data in which the aim is to evaluate the role of tranquilizing drugs in the recent decline in State mental hospital populations, a decline which has occurred in California as well as in many other States.

For the period from July 1, 1955, through December 31, 1957, additional information (i.e., additional to

that routinely obtained for all patients) on drug therapy was recorded for each patient in the State system who had received treatment with drugs. Name of the drug, total amount of drug administered, number of days on drug, and the reason for stopping drug treatment were among the additional data recorded. By looking at significant subgroups—for example, first-admission male schizophrenics between the ages of 25 and 44—the investigators hoped to be able to draw certain conclusions about shortened periods of hospitalization and their association with drug therapy. Data concerning the details of drug dosage during the period of the study now are being analyzed.

Epstein stated that investigators participating in this study are "painfully aware" that the data involve a variety of physicians, drugs, settings, and timings of drug treatment in relation to admission, as well as a variety of discharge policies among the 11 different hospitals. Despite such problems, the data do provide some reflection of what may be associated with drugs as they are used in a hospital system.

B. *Summaries of Presentations of Research Methods*

Specifying and predicting the effects of treatment were of prime concern during this part of the meeting. In considering these problems, papers were presented in which attempts were made to summarize the evidence on characteristics of patients that may be related to response from treatment, and aspects of behavior that are expected to change with treatment. The methodological problems associated with the selection of techniques for the collection of data, the classification of patients, statistical analysis, and the measurement of change in patients were also taken up in separate sections of this part of the meeting.

In addition to reviewing the information and methods on these matters, the participants were asked to consider and to suggest approaches that might be of help in clarifying the effects of new methods of treatment.

1. Predictor Variables

Clinical history was discussed by Bernard Glueck. Although several clinical and social variables have demonstrated some general predictive value in studies of response to treatment, Glueck observed that the search for factors of specific value has not been fruitful. He reviewed some of the variables of clinical history that have been linked to response to insulin therapy, electroshock, and lobotomy, and commented on the scarcity of such information in relation to treatment with drugs. His major criticism was aimed at the continuing lack of a common language to describe psychiatric conditions. Following a description of Q-sort techniques, which he and his associates are applying to this problem, Glueck suggested that Q-sort methods provide a means of standardizing language and making comparable the findings from different clinics or hospitals.

Sociological variables were covered by Leonard Pearlin. Arguing for greater specificity in this area, Pearlin observed that generic variables such as social class, age, and sex are too global to be of much value in understanding the relations among variables. The need, he maintained, is for greater emphasis on description of the social context—i.e., the family, the community—and a descriptive system in which the "social characteristic in context" is the unit of analysis.

The role of personality in the prediction of response to treatment was the topic of Seymour Fisher's paper. Fisher's criticisms of previous attempts to relate personality variables to treatment response also noted the failure of investigators to place sufficient weight on the influence of situational factors. Fisher did emphasize, however, the need to derive variables from theory, which probably will be more complex than variables previously used in such studies, but which may be less subject to the influences of sample variations and situational differences. He was explicit in pointing out several difficulties in applying personality measures to prognosis. These were (*a*) the curvilinear rather than linear relationship which usually exists between a quantity of a given personality trait and level of adjustment, (*b*) the significance of a given trait as a function of social class, e.g., high level of aspiration may be a virtue in middle class groups, but it has a different meaning for upper class groups, (*c*) the relative importance and role of a given trait in personality differs among people, e.g., passivity in one personality is a central problem, aggression is a central problem in another, (*d*) the kind of social context in which the trait is manifested affects the possible significance of the trait with regard to prognosis, e.g., a highly aggressive patient returning to a highly agressive wife can have a different outcome from the same type of patient returning to a passive wife.

Fisher provided examples of several personality dimensions which might overcome some of the difficulties of the past, but which will also entail some complicated methodological questions. His point, however, was that the emphasis in the selection of prognostic index should shift from overreliance on existing empirical techniques to concern with the derivation of theoretically meaningful variables.

2. Data Collection and Analysis

Methods for dealing with descriptive population variables were discussed by Samuel B. Lyerly and Dean J. Clyde.

Lyerly, in a paper entitled *Interview Data: Coding, Scaling and Selection of Potentially Useful Variables,* emphasized the differences between hospitals and communities that affect the collection and analysis of data, the characteristics of information that are essential

to statistical analysis of data, and the importance of ensuring that information is comparable from subject to subject. With regard to coding, he discussed data and classification systems, the role of the pilot study, ways of handling "free responses," and the application of simple mathematical procedures to the derivation of patterns from profiles. Problems of weighting, suggestions for dealing with "does not apply" responses, and the application of different types of validity models were also considered.

Clyde's paper, *Multivariate Problems: Clustering Variables and Classifying Patients into Types,* focused on the role of multivariate models in drug research. He described the following three approaches, and presented examples of the application of each: (*a*) analysis of covariance; its use was exemplified in a study in which control was required for the differences among patients, prior to treatment, with regard to the severity of their illness; (*b*) factor analysis which was used, in the example presented, to reduce a large number of items in a rating scale to two independent dimensions, and which served thereupon to clarify the composition and meaning of the instrument; and (*c*) discriminant function, which was applied to a problem of separating groups of patients on the basis of their differential response to drug treatment. The relevance of the latter procedure to the problem of etiology was also considered.

In discussing these papers, Goldine Gleser elaborated upon several ways to separate subjects into meaningful groups. Three statistical models for accomplishing this kind of separation were described. She stressed that the state of knowledge in the field is not sufficiently advanced to permit one to predict the best way of classifying patients for any given study, but pointed out that study of the outcome of such empirical separations can yield hypotheses which can then be cross-validated in other studies.

3. Methods for Measuring Improvement

Norbert Freedman and David Mann described the manner in which they are attempting to measure psychopathology and social behavior. Emphasis within the Kings County Clinic is on the psychiatric rating scale approach, and in their community studies, emphasis is on a "naturalistic" approach. They have, through preliminary analysis of their psychopathology ratings, identified factors that improve with drug treatment and that allow one to predict response to drug treatment. On social behavior, development of an extensive interview schedule covering family history, work history, and social pathology was described by Mann. He also discussed in some detail their coding procedures, the progress of their approach to studying the "typical day in the patient's life," and the dimensions of classification that have been derived from the social data and that will help to define "social remission." It was pointed out that the definition of social remission is one of the central aims of the project.

The details of a rating scale for measuring the improvement of outpatient psychotics treated with drug and placebo were discussed by Jordan Lawrence. The scale, which is completed by a psychiatrist or psychologist and a social worker following an interview with the patient, has three sections, one covering major psychopathology, one describing neurotic symptoms, and one concerned with social adjustment. Lawrence reported that the more reliable items in the scale have been factored and have yielded tentative dimensions of "schizophrenia" and "depression." He also indicated that the three subscores and the total score have been found to discriminate well between conditions of prerelapse and relapse, but noted that further, better controlled validational studies need to be done.

Progress on the development of a set of inventories designed to assess clinical and social adjustment was reported by Martin M. Katz. He noted that the instruments are based on the need to integrate two points of view, the patient's and the relative's, in assessing the adjustment and performance of the patient. The inventories represent attempts to obtain objective estimates of (*a*) the level and quality of the patient's psychopathology and social behavior, (*b*) the amount and kind of home and free-time activity in which the patient is involved, and (*c*) the patient's and the relative's level of satisfaction with the patient's functioning in clinical, work, social behavior, and free-time activities. A validity study was described in which the relatives' assessment was shown to agree highly with the psychiatric assessment (based on intensive clinical study of the patient) with regard to the presence of psychopathology, and the extent of home, social, and free-time activities of the patient. Several trends in the data were noted: the relative can provide accurate, objective information on certain matters; the sheer quantity of activity as reported by patient and relative reflects the level of adjustment; the relative's level of expectations at the time of assessment correlate highly with adjustment. More detailed study of the composition of the instruments and their applicability is in progress.

Mark Lefton described his implementation of the interview schedule approach in the assessment of several key areas of behavior. Separate schedules were designed for the patient and the relative. The variables of prime interest in Lefton's assessment of community adjustment are social participation, work performance, psychological functioning as measured by a relative's ratings on a list of psychopathological index, performance as a homemaker, and measures of the relative's expectations and tolerance of deviation. He reported that several measures have been found to discriminate between patients who were returned to

the hospital within 6 months and those who remained in the community, as well as between patients who function well and those who function poorly in the community.

SUMMARY OF CLINICAL RESULTS

The results from the separate projects that pertain to clinical matters in the community care of patients on drugs are summarized below.

1. The Effectiveness of Maintenance Drug Treatment

(*a*) Gross' conclusion that it is necessary to maintain the large majority of chronic schizophrenics on drugs following release from the hospital is based on the differential relapse rate in his drug and placebo groups. The rate of relapse during a period of 8 months was three to four times greater in the non-drug group than in the group on drug maintenance.

(*b*) The hospitalization rate in the Kings County Clinic placebo group was 28%; in the chlorpromazine group it was 5%. The difference is statistically significant.

(*c*) Improvement at 6 months as reported by Engelhardt for the chlorpromazine group was 46%, for the promazine group 33%, and the placebo group 8%.

(*d*) 27 of 35 patients have been returned to work during the course of 1 year in the Manhattan Aftercare Clinic day-care program. Only 7 of a group of 33 patients who were returned to the hospital are now working.

2. Patient Factors Related to Drug Effectiveness

(*a*) Possible predictors of relapse when drugs are discontinued. On the basis of his experience with chronic psychotics in the outpatient clinic, Gross lists the following factors as related to relapse when drugs are discontinued:

(*1*) Age: Patients under 30 and over 50 are more likely to relapse when drugs are discontinued;

(*2*) Previous history of relapse within 2 years following discharge;

(*3*) Intolerant family; and

(*4*) Failure of the patient to assume a responsible role in the family.

(*b*) Predictors of improvement with drug treatment [Engelhardt].[1] Preliminary results indicated that severe initial disturbance in thought processes, and great concern over being mentally ill are related to improvement with drug treatment.

[1] The source of the evidence is indicated in brackets.

3. Differential Effects of Drugs

(*a*) Several drugs were used in the Springfield State Hospital study but the study was not designed to compare drugs; results, therefore, cannot be attributed to any single drug [Gross].

(*b*) The Kings County group found chlorpromazine to be more effective than promazine in preventing rehospitalization [Englehardt].

(*c*) The Manhattan Aftercare Clinic research group was not particularly interested in the comparison of drugs; this group tends to rely chiefly on chlorpromazine [Kris].

4. Possible Detrimental Effects of Long-Term Administration of Drugs

(*a*) No differences between drug and placebo groups following prolonged treatment are found on the Porteus Maze test: a performance measure which purports to assess intellectual functioning [Engelhardt].

(*b*) Pasamanick's informal report of a study at the Columbus Psychiatric Institute stated that chlorpromazine was found to have no deleterious effects on mental functioning as measured by a number of psychological performance tests.

(*c*) Social dysfunctioning in the chlorpromazine group was less than in the placebo group as reported by the family on a behavioral checklist devised by the Engelhardt group.

(*d*) Kris reports no accidents at work or obvious decrements in work or home adjustment in patients on prolonged maintenance treatment.

5. Time Considerations in the Efficacy of Drug Maintenance

(*a*) The optimum time for cautious withdrawal for chronic patients who were stabilized on drugs prior to research may be during the second year of drug maintenance treatment [Gross].

(*b*) Questions as to whether improvement occurs only in the early stages of drug maintenance treatment with certain types of patients, only in the later stages with other types, and whether it is a continuing process, are being studied by the Kings County group [Engelhardt].

6. Dosage Recommendations and Side Effects

(*a*) The average maintenance dose reported by Engelhardt is less than 250 mg/day of chlorpromazine.

(*b*) Kris finds one evening dose to be optimum and less likely to result in side effects during the day.

(*c*) Side effects have not posed a major problem in the studies reported.

7. The Management of Relapse

(*a*) Kris reported that the most effective procedure with relapsed patients was to prescribe 400-800 mgs/day of chlorpromazine for a few days, followed by return to a maintenance schedule. Patients in the day-care program are released from treatment within 2 to 6 weeks; the average is 2 to 3 weeks.

(*b*) Patients previously on a maintenance dosage, who relapse after being shifted to a placebo, recover fairly well with resumed medication, and do not require hospitalization [Gross].

8. The Drop-out Problem

(*a*) In Gross' situation, where earlier hospitalization had been followed by long-term contact with patient and relatives in a hospital-affiliated aftercare clinic, the drop-out rate was very low (on the order of 10%).

(*b*) In the study at Kings County, at 3 months 65% of the group were still in the study; at 1 year 35%. Drop-out rate was roughly equal for all treatment groups. Drop-outs included both failures and successes, although, early drop-outs were usually failures.

9. Changes in Discharge Practices

(*a*) The likelihood of discharge from the hospital is in part a function of the availability of facilities and resource people in the community [Greenblatt]. It is likely then that with the increase in transitional type facilities and greater reliance on drug maintenance, patients will be returned more quickly and in even greater numbers, to the community.

(*b*) Patients are already being discharged much earlier than previously. Thirty-two percent of the patient group hospitalized in California in 1949 were still in the hospital *three* years later. Of a group hospitalized in 1956, only 19% were still in the hospital *one* year later [Morgan].

10. Limitations on the Generality of Findings

The sample limitations placed on the generalization of findings in the conference studies are worth indicating.

(*a*) The Kings County investigation, because of its need for extensive information on the social and family behavior of the patient, has confined itself to a group who have relatives as informants. They hope later to extend the study to the more "isolated" schizophrenic in the community.

(*b*) Gross' outpatient group was confined to chronic psychotics who were well-stabilized on a maintenance schedule for a period of at least 6 months. This group, it was pointed out by the investigator, did not include acute schizophrenics and was not representative of the general clinic population.

(*c*) The Manhattan Aftercare Clinic group were representative of "ex-hospitalized patients who relapse some time following their return to the community and would ordinarily be returned to the hospital." They were composed, however, of patients who had been cooperative enough to report to the research unit for followup treatment the day after their initial release from a State hospital, and had maintained clinic contact.

(*d*) In comparing home care and pharmacotherapy with hospitalization in an acute population, Pasamanick made clear the limitations of his sample. Since this program had not started at the time of the conference, the difficulties in equating the samples were discussed. The groups to be compared will represent acute psychotics, not requiring emergency hospitalization but sufficiently ill to be hospitalized, a group, in other words, that ordinarily would have been hospitalized under the routine hospital procedures existing prior to the study.

PROBLEMS IN MEETING THE REQUIREMENTS OF RESEARCH DESIGN

The studies which were carried out in the community pointed up several problems that are somewhat peculiar to the accomplishment of well-designed research in this setting. Since several of the investigators had already worked out some of these problems in the course of their studies, the discussion helped to suggest some solutions, and to underline the difficulties still to be solved in conducting controlled clinical research.

1. Random Assignment of Patients to Experimental and Control Treatments

Since the studies were designed to compare two or more treatments in a controlled manner, all investigators assigned patients randomly to treatments. From a clinical standpoint, when a new treatment is being compared with an older, more traditional approach (which would represent the control treatment), there is little objection from an ethical standpoint to the random assignment procedure. None of the study patients in this situation is really being deprived of adequate treatment. When placebos are used for control, then controversy can emerge with regard to ethical considerations.

This topic has been the subject of intense discussion at many meetings. There are fairly sound arguments on both sides which need not be repeated here, but it would seem that the particular situation and the investigators own leanings form the basis for deciding

how a study is done. There are some strong feelings that in the absence of knowledge concerning the effectiveness of a treatment method using a drug, the use of a placebo represents an appropriate treatment. At any rate this problem did not appear in these studies, and it did not inhibit the investigators or limit the type of research carried out.

Somewhat akin to the ethical problem, however, is the reluctance of clinicians to assign acutely ill patients to treatments on a random basis, for fear that the illness will get out of hand. The clinician obviously has to proceed along the safer path even if he may feel strongly about the potentiality of a new treatment. Where research is concerned, however, it is unfortunately necessary that exceptions not be made without running the risk of limiting seriously, if not ruling out entirely, any generalizations that can be drawn from the investigation. In the context of this restriction, and prior to the initiation of a study, there is a tendency to see only the negative or dangerous situations that may arise under such conditions. On the other hand, it was quite clear that the long-term value of the results arising from these "controlled" studies, in terms of their contributions to the effectiveness of clinical practice in general, outweighed the importance of the small number of expected failures. The fact that it was not necessary to "break" the assignment procedure except in the rarest of circumstances, suggests that the dangers tend to be exaggerated in the absence of information on the problem. Placebos were used in Gross' and Engelhardt's studies without the occurrence of any serious problems. The results of these studies have helped decidedly to clarify the effects of drugs in maintaining patients in the community, and, in addition, have provided evidence on the relative efficacy of two drugs in making this possible. Further, the results can now be generalized to the fairly large populations of patients, which the samples of patients represented—generalizations that would not have been possible if the random assignment procedure were not followed.

Kris provided a graphic example of how the requirement of randomness can sometimes help overcome the reluctance of clinic and family, and demonstrate unexpected advantages of a new treatment. In the clinic situation, it was sometimes necessary to assign patients to day-care and to drugs for whom, on the basis of the family's demand and the acuteness of the patient's condition, rehospitalization would have been both clinically more convenient and more acceptable to the family. The patients who were not hospitalized despite the family's demands responded well to the combination of drugs and day-care. Aside from the values noted in conforming to the requirements of research design, these incidents broadened Kris' concept of the applicability of the day-care approach. If, for example, very acute conditions can be treated with this approach, then many of the secondary advantages that accompany the avoidance of hospitalization are made possible. These patients were back at work in 2 to 3 weeks and their confidence in their own capacity to recover fairly rapidly from these episodes was obviously bolstered.

2. Comparison of the Effectiveness of a Treatment in Two Hospitals

In comparing a given treatment in two hospitals, the problems in designing research can obviously be very different from those faced in the community studies. It might be worth noting that the Massachusetts Mental Health Center was not entirely successful in its attempt to separate drug from milieu effects. A number of concessions had to be made to the practical requirements of the situation; consequently, the results have to be viewed as highly tentative. The study, which represented an exploratory comparative approach, pointed up, however, several practical problems that have to be solved or endured in conducting this type of research.

(*a*) The need to transfer patients from one hospital to another resulted in a number of unexpected problems. Greenblatt described in detail the "transfer trauma" and its effects on patients and the hospital. The period necessary for restabilization of the patient in a new setting can be very disturbing to the hospital and to the research program.

(*b*) The criteria of improvement may not be comparable in the two settings. For example, when discharge was used as a criterion, the two hospitals differed markedly in practice. Patients in one hospital, who on objective rating scales appeared the same as the patients in the other, were discharged more quickly, simply as a function of the availability of community resources.

(*c*) The time required for a treatment to demonstrate its effects, if any, may vary with different treatment procedures. At 6 months, the milieu effect apparently added nothing to the extent of improvement accomplished with drugs; i.e., the rate of improvement in the two hospitals was roughly equal; at 18 months, the interaction of milieu and drugs began to produce some positive effect over and above drugs alone.

RECOMMENDATIONS ON METHODOLOGICAL ISSUES

1. Sample Description

There is little uniformity in the manner in which investigators describe the sample of patients in their study. These deficiencies in description make for

obvious difficulties in estimating the generality of results, as was previously noted. The minimal information required for good reporting on new drug treatments was indicated by Cole, Bouthilet, and Ross.[2] The problem of how to specify and describe the patient's clinical condition for research purposes is still far from being solved. Glueck maintained that the use of clinical diagnosis is inadequate; the subjectivity involved in the present diagnostic system does not permit results from one study to another to be compared. He suggested adopting a more objective atomistic approach, through Q-sort techniques, to remedy the situation in comparative studies of treatment. Whether the Q-sort, with its limitations, is the most appropriate approach to deal with this problem is still very much in question. There are other similar quantitative approaches that might be more applicable to the diagnostic situation[3] but the problem is obviously a real one, and until investigators are willing to devote more effort to this phase of research, the specificity of results for certain patients, and the opportunities for generalizing to patients in other settings will continue to be a tenuous procedure.

2. Predictor Variables

It is quite clear from previous work that attempts to predict response to treatment on the basis of factors in a patient's background have been rarely successful, except for a few variables, such as rapidity of onset and quality of affect, which have been consistently related to outcome.[4] Both Pearlin and Fisher agreed that part of the problem has been the tendency to oversimplify the task and to rely too heavily on those variables which require the least effort to measure. Pearlin's point—that social variables such as age and sex role have to be placed in context to be useful as predictors—was directed towards this deficiency. The fact that these generic variables take on a variety of meanings as a function of the type of family, the social class, and the type of community, increases the difficulty of putting them to use as predictors or attempting to relate them to other aspects of treatment. Fisher, also, sees these complex relationships of variables and situations as confounding problems in attempts to predict from the more simple personality variables. The need to derive configural dimensions based on personality theory is an attempt to reduce the extensive reliance on established, but very limited, techniques in this area.

We believe that the same criticisms can, of course, be directed toward the other side of the problem, i.e., with respect to measuring the outcome of treatment. It can be argued, for example, that success in finding predictors will have to await more careful development of criterion measures of acute and long-term response to treatment.

The solutions offered at the conference do not help to simplify these problems but they do point the way toward clarifying some past difficulties. There is little need to look for specific predictors to treatment response when the treatments themselves lack specificity. The differential action of the newer drugs are contributing toward greater specificity in psychiatric treatment; consequently the need to search diligently for specific predictors becomes more pressing. This development obviously requires more thinking and more diversity in research approach.

3. Analysis of Interview Data

Lyerly, in emphasizing the heterogeneity of home settings and the need to rely on nonprofessional observers, pointed out some of the reasons which make research in the community more complicated than similar studies carried out within hospitals.

Despite the fact that, in most instances, the investigator is limited to the interview approach, the basic requirements of collecting data, (i.e., objectivity, quantifiability and completeness) can be approximated very closely if not entirely. The coding of open-ended responses is a key problem here, but it is clear that for the most part it can be adequately dealt with, if care is given to the comparability of language and the realiability of judgments.

As will be discussed in the section on measuring change, the interview approach is fundamental in the exploratory phase of study, but the problems of comparability, reliability, etc., make it one of the most difficult techniques to handle in research. Coding, weighting, and validity require in most cases the advice of experts, and there is no substitute for careful thought on the nature of the information needed, the best manner of obtaining it, and the way it will be handled in the construction of the schedules. We have come to the conclusion, however, that the most prominent defect in such studies is not in the structure of such schedules but in the content of the protocols. Investigators who construct interview schedules often fail to realize that the same care that goes into the framing of hypotheses for an experiment must go into the planning of questions for an interview schedule, which is to be used in research. As was evi-

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[2] Recommendations for reporting studies of psychiatric drugs. *Public Health Reports* 72, 1957, 638-645.

[3] Katz, M. Problems in evaluation of effectiveness of transitional facilities. In Greenblatt, M., et al. (editor). *Mental patients in transition. Springfield, Ill.: Charles C Thomas, 1961.* Pp. 118-126.

[4] Zubin, J. Role of prognostic indicators in the evaluation of therapy. In Cole, J. O., and Gerard, R. W. (editors). *Psychopharmacology: Problems in evaluation.* Washington, D. C.: National Academy of Sciences—National Research Council, 1959. Pp. 343-355.

dent in both Lyerly's and Clyde's presentations, the techniques are for the most part available for the statistical analysis of these data. There are, in other words, no technical reasons to avoid the interview schedule approach. The problems seem to lie in the context area and in the lack of conceptual clarity which has limited greatly the contribution which the interview method has made in psychiatric research.

4. Multivariate Analysis and Methods of Classifying Patients

The differential influence of many variables on the outcome of treatment, poses questions as to the manner in which selected variables interact to create an effect, and ways of reducing large numbers of variables to a finite number of meaningful factors. For these questions, the techniques for statistical analysis are available, and they are sufficiently adaptable to meet most of the problems encountered in this field.

Lack of clarity in statistical methodology still seems to reside in classifying types of patients. This topic was only covered briefly at the conference. The problems in specifying types of patients who differeptially respond to treatments can be handled, as Clyde and Gleser pointed out, through discriminant function and possibly through factor analytic techniques, but the assumptions that have to be met, and the approaches that need to be tried, still make the process highly complicated. Gleser pointed out that a great deal of care has to go into the selection of the appropriate statistical model for the problem at hand. Some of the controversy that arose in the discussion of applying the Q-technique exemplified the sharp lack of agreement on this subject. Because of the increasing concern about the classification of patients and dissatisfaction in research situations with the present diagnostic system, the content and statistical problems here obviously should be considered more intensively and searchingly.

5. Measuring Change

(*a*) *Defining adjustment.* The investigators who reported on methods at the conference, indicated an aim: the development of techniques to assess the social functioning of patients in the community. They also tended to agree fairly closely on the general aspects of social functioning that have to be examined in order to arrive at meaningful assessments. All indicated, for example, that they attempted to cover such matters as work and behavior at home. They differ on the relative amount of importance, with regard to overall assessment, that they attach to different factors. They also differed in the extent to which they are ready to commit themselves to a definition of "social remission" or "social adjustment." The extent of commitment to a definition, it seems to us, determines how each investigator will approach the collection of data. The naturalistic approach, exemplified by the open-ended interview, is used by the Kings County group and indicates their interest in collecting large amounts of descriptive data to be used to arrive, eventually, at an explicit definition of social remission. Lawrence's and Gross' reliance on social workers' judgments of the patient's functioning in the social sphere implies that they already have a fairly clear idea of what constitutes social adjustment. The other approaches would fall somewhere in between. Lefton is, however, the only investigator who is now attempting to get control data, that is, comparable data on the functioning of normals in various social activities. Katz, although in agreement with the other investigators concerning the importance of descriptive reports of patient activities, is committed to emphasis on subjective (attitudinal) elements in his definition. This tends to guide the method of approach (i.e., to measure the subjective concept, "satisfaction with patient functioning," methods had to be devised that were based on the discrepancy between expected and observed activities of patients).

(*b*) *Source of data.* What also seemed clear is the tendency for all of the investigators to rely on a close relative as a prime source of information. Although each investigator is in some way attempting to ensure adequate coverage by leaning on clinical judgment, either for supplemental data or as a criterion, it is evident that the source to be relied on primarily is a relative. The reasons for depending on the relative are not very difficult to understand. The expert who sees the patient or relative for only a brief interview has to rely on the informant's report to arrive at a judgment. There appeared to be general agreement that the expert's experience in assessing psychopathology does not necessarily result in accurate prediction of the patient's social functioning in the community. The more direct approach is to go to the source of such information, the patient and his family. The patient, however, as a reporter of his own behavior is not expected to be very reliable; too many extraneous but important influences act on the accuracy with which he will report. When chronic psychotics are involved, for example, the patient fears that he may be returned to the hospital and, in many cases, he distorts reality pathologically. The relative is also open to biases of this type; he may be "sick" also, and these considerations have tended to make investigators in the past somewhat cautious in accepting relatives' reports as a basis for objective assessment. The Barrabee-Finesinger Scales,[5] for example, although heavily dependent on the relative for infor-

[5] Barrabee, P., et al. A normative social adjustment scale. *American Journal of Psychiatry* 112, 1955, 252-259.

mation, relies finally on the judgment of the clinician in sorting out these biases in the assessment of the patient's functioning.

Except for the Lawrence-Gross approach, the other instruments described at the conference stay much closer to the relative's actual report than has been the practice in the past. They avoid placing undue emphasis on the clinical evaluation of the relative's report. In essence the investigators appear to assume in common that despite certain biases in the relatives, they are capable of reporting accurate information.

The problem is primarily one of technique, of applying the most appropriate interview or psychometric approach to obtain information and to assess its accuracy. In this respect, it is noted that Mann emphasizes openness of interview, completeness in description from the relative, and respect for what is "salient" in the relative's picture of the patient. Katz, on the other hand, relies more on presenting the relative with a structured comprehensive range of behavior, and on the relative's accuracy in reporting separate types of behavior, than on the relative's ability to make complex judgments about the patient's feelings or behavioral patterns. The problems with the relative's bias then has not been avoided, rather it has been accepted in the acknowledgment that he is really the most appropriate, if not the only person available to the investigator who can report on how the patient is acting and what he is actually doing in the community. When the traditional criterion of assessing patients (that is, the extensive study of the patient and his situation by psychiatrist and social worker) is used as a means for determining the relative's accuracy in reporting the patient's level of psychopathology [Katz], then the relative is found to be more reliable in this respect than previous information may have indicated.

It should be noted, however, that in all of the approaches that were described, the patients' reports figure in the assessments, but again, depending on the method, may carry different weight. Both Lefton and Katz are placing more emphasis on the patient's report than the other investigators.

(*c*) *Methods of measurement.* Several types of methods were presented and their differences warrant some consideration. How a method is selected is guided, as was noted earlier, by the kind of information the investigator is after, how difficult it is to obtain, who it is obtained from, and what the investigator intends to do with the information. When the subject is social functioning, it is obvious that the immature state of the field and the lack of agreement with regard to this concept makes for varied approaches to the problem.

The characteristics of the approaches described at the conference are listed in the table.

Methods for Collecting Data on Adjustment of Patients (Described at the Conference)

Methods and Their Characteristics	*Techniques for Summarizing Data*
INTERVIEW Administered and recorded by trained interviewer, usually a social scientist or social worker.	(1) *Coding:* Responses can be pre-coded (categorized beforehand), which eliminates the need for trained raters.
Unstructured (open-ended). General topics are specified, questions are unspecified, provision for open-ended responses.	Open-ended responses require codes that entail specially trained raters.
Structured. Questions are specified; varying degrees of providing for categorization of responses; some open-ended responses are usually provided for.	Responses to single items or to clusters of items are used to derive descriptive indexes or measures of functioning.
	(2) *Judgmental Rating Scales:* Experts rate behavior on the basis of information gathered in the unstructured or structured interview.
INVENTORY Administered by psychometrist, but respondent completes forms; includes list of items that require categorized responses.	*Scoring:* Measures of various aspects of behavior are derived by summing responses according to an a priori scoring system.

As noted in the discussion on definition of adjustment, the more structured the concept in the investigator's mind the more structured is the approach. The open-ended interview implies the least conceptual structure. The other two imply more structure but are selected for different reasons, and differ in the types of data collected and in the analysis of data. The structured interview and psychometric inventory are closer in these respects than either is with the unstructured interview. The amount of information that is used in developing indexes is less than with the open-ended interview, and the nature of the information collected is more consistent from case to case. The aims, when the structured techniques are used, are also likely to be made more explicit than is the case when the open-ended interview is used. The success of investigators who use the structured approaches in accomplishing these aims is usually easier to assess also. It is obvious that the merits of these approaches for different research situations can be debated. Our impression, which may or may not agree with other investigators', is that a fairly balanced set of approaches is being tried out to deal with different aspects of the problem. In regard to the other participants in the conference, the writers note that:

1. All are interested in what social adjustment is. It is salutary, therefore, to have the naturalistic ap-

proach as well as the exploratory aspects of the other approaches.

2. All are interested in identifying the best people to collect such data. Therefore, it is valuable to have social science interviewers and social workers working on the interview problem and, at the same time, to have continuing attempts to get direct reports from the relatives through tests.

3. Neither the need for more exploratory research in this area nor the pressing need to develop more formal measures to assess adjustment are being neglected.

SUMMARY AND CONCLUSIONS

This report summarizes the proceedings of a research conference on drugs and community care, abstracts the more important clinical results that were reported, and makes some recommendations on issues of methodology that grew out of the conference's discussions.

The authors see four major conclusions that can be drawn from the work presented:

(1) Reassuring evidence now exists that controlled research can be successfully carried out on such problems. The studies described can be used as models for the evaluation of other innovations in community treatment.

(2) Results, of clinical importance, on the value of drugs and other community-based treatment procedures are now available.

(3) With the increased diversity of psychiatric treatments, the problems of predicting and specifying the effects of different treatments become more pressing. There is a need, therefore, for more experimentation with various ways of classifying patients, and with new ways of looking at some of the older approaches to prediction. Of the several methodological problems discussed at the conference, this one seemed to be most urgent, not only in relation to the resolution of statistical issues but primarily with regard to defining significant variables in order to help classify patient types meaningfully, and to predict response to treatment.

(4) Several methods for assessing clinical change and adjustment of patients who are living in the community are now available for more intensive study and wider use.

PAPERS READ AT THE RESEARCH CONFERENCE ON DRUGS AND COMMUNITY CARE, NATIONAL INSTITUTE OF MENTAL HEALTH, WASHINGTON, D. C., SEPTEMBER 1960.

Clyde, D. J. Multivariate problems: Clustering variables and classifying patients into types.

Engelhardt, D. M. A study of ataractics in outpatient schizophrenics.

Fisher, S. Personality: Predictor variables.

Freedman, N., and Mann, D. A study of ataractics in outpatient schizophrenia: Methods of psychiatric and social behavior assessment.

Glueck, B. Clinical history: Predictor variables.

Greenblatt, M. Drugs and social therapy in chronic schizophrenia.

Gross, M. Termination of treatment with ataractic drugs.

Katz, M. Methods for measuring clinical and social adjustment.

Kris, Else B. Drug therapy in a daycare facility for relapse control.

Lawrence, J. Termination of treatment with ataractic drugs: A rating scale for psychiatric outpatients.

Lefton, M. Home versus hospital care for schizophrenics: Methods for assessing social performance and psychological functioning.

Lyerly, S. Interview data: Coding, scaling and selection of potentially useful variables.

Morgan, R., and Epstein, L. The effect of ataractic drugs on hospital release rates.

Pasamanick, B. Home versus hospital care for schizophrenics.

Pearlin, L. Sociologic characteristics as predictors of treatment outcome.

Volume and Serial Numbers for *Bulletin*

Starting with the next issue of the Psychopharmacology Service Center *Bulletin*, volume and serial numbers will appear on the cover. Without such numbers on an irregular publication such as ours, as several of our readers have noted, it is impossible to know whether one has received all issues. We shall consider all issues prior to 1962 as Volume I; Volume II, No. 1, will commence with the first issue in 1962. The issues considered to make up Volume I are: in 1959, January, April, June, July, and November; in 1960, March, April, July, September, and December; in 1961, January, March, July, September, and December.

Early Clinical Evaluation of Drugs

As announced in the January 1961 issue of this *Bulletin* (p. 3), the National Institute of Mental Health has established a program of special grants for early clinical investigations of psychiatric drugs. The primary purpose of these grants is to broaden the present scope of early clinical trials of promising new compounds, and to make it possible to screen more new drugs for effectiveness in treating psychiatric disorders.

Since the grants were established in November 1960, eleven research units have been carefully selected to conduct early clinical evaluations of drugs. The units, the principal investigators, and the grant numbers are listed below.

MYP-5098 Jean Delay, M.D.
Centre Psychiatrique Ste. Anne
Paris, France

MYP-5093 Herman C. B. Denber, M.D.
Manhattan State Hospital
New York, New York

MYP-5090 David M. Engelhardt, M.D.
Downstate Medical Center
Brooklyn, New York

MYP-5089 Harry Freeman, M.D.
Medfield State Hospital
Medfield, Massachusetts

MYP-4669 Arnold Friedhoff, M.D.
New York University Medical Center
New York, New York

MYP-5144 Leo Hollister, M.D.
Veterans Administration Hospital
Palo Alto, California

MYP-5121 John Kinross-Wright, M.D.
Baylor University, College of Medicine
Houston, Texas

MYP-5096 Sidney Merlis, M.D.
Central Islip State Hospital
Central Islip, New York

MYP-5106 Burtrum C. Schiele, M.D.
University of Minnesota
Minneapolis, Minnesota

MYP-5202 Heinz E. Lehmann, M.D.
Verdun Protestant Hospital
Verdun, Quebec, Canada

MYP-4665 Barbara Fish, M.D.
New York University Medical Center
New York, New York

Another group of investigators held grants from the National Institute of Mental Health for the same purpose prior to the instigation of this special program. These investigators are:

MY-1665 Sidney Malitz, M.D.
New York State Psychiatric Institute
New York 32, New York

MY-3701 Robert G. Heath, M.D.
Tulane University School of Medicine
New Orleans, Louisiana

MY-3712 William P. Boger, M.D.
Norristown State Hospital
Norristown, Pennsylvania

It is hoped that these research units will provide a balance between careful observational studies and small-scale controlled and semi-controlled studies, being flexible enough to tailor the research design to fit the drugs and patients under study. The individual investigators will be largely responsible for the choice of drugs to be studied, and for obtaining the drugs. The staff of the Psychopharmacology Service Center will provide the investigators with appropriate assistance upon request.

An announcement describing the program may be obtained by writing to Dr. Jonathan O. Cole, Psychopharmacology Service Center, National Institute of Mental Health.

*Serotonin**

Determination, Metabolism, Pharmacology

Some Biological Aspects

B. Maupin **

"An hour of synthesis is worth years of analysis."
CLAUDE BERNARD

In 1948, Rapport and his collaborators announced that the vasoconstrictor factor of serum, called serotonin,[1] was 5-hydroxytryptamine, a relatively simple chemical compound whose synthesis was soon accomplished. In 1952, Erspamer identified it with enteramine, an organic compound which he had isolated from the mucous membrane of the gastro-intestinal tract. Zucker indicated that serotonin was carried by the blood platelets, but did not originate from them. Then it was learned that serotonin, or enteramine, or 5-hydroxytryptamine, was produced in abundance by tumors of the chromaffin system; that it was present in nervous tissue under normal conditions; that it acted as a synergist with certain tranquilizers; and that lysergic acid was a serotonin antagonist.

These joint and single discoveries did not exhaust the interest displayed in serotonin, first in the United States and Italy and then all over the world, in numerous publications on the subject. On the contrary, new questions arose and are still being debated. Is serotonin a hormone? Is it a chemical mediator? Does it have therapeutic value?

In spite of the many unanswered questions, these newly developed concepts seemed important enough to us to merit critical evaluation. The present review is based, among other sources, on important reviews by Erspamer, Page, Jeanne Levy, and Marjorie Zucker, and on the proceedings of the London Symposium, the New York Academy of Sciences, the Société de Médecine Militaire Française, etc. If we do not succeed in justifying Claude Bernard's adage, quoted at the beginning of this paper, we shall plead as an excuse the difficulty of the subject, "for there are few substances for which the accumulated facts seem so incomprehensible." [143b]

Our long-standing interest in blood platelets, cells involved in hemostasis, will explain to the reader the emphasis placed in this paper on their relations with serotonin. On the other hand, we will only mention briefly the relations between serotonin and the central nervous system; the subject should be developed extensively by itself.

* Translated from the French by Charlotte Waldo, under Public Health Service Grant No. 3M-9163, and by Denise Oudot, staff member of the Psychopharmacology Service Center. Permission to publish this translation has been cordially granted by the author and the journal in which the article appears in the original: *La Biologie*, 1960, 49, 1, 75-164.

** Specialist for Military Hospitals; Sc. D. (Army Center for Transfusion and Resuscitation. Director: Méd. Gén. J. Julliard, 1, rue Raoul Batany, Clamart, Seine.)

[1] We remind our readers that the name "serotonin" was suggested by Rapport, Green, and Page, with the collaboration of Corcoran (1948 [205]). The term has flourished. Page is now humorously claiming that he can hardly recognize his own child.

CHAPTER I

Chemical Nature

Identification and Determination

Chemically, serotonin has been identified as 5-hydroxytryptamine (5-HT). (See Table 1) [2] After this de-

[2] A recent decision of the Commission on Nomenclature of Organic Chemistry of the International Union of Pure and Applied Chemistry prefers the term hydroxy-5-tryptamine. But the literature which we are reviewing has made such wide usage of the name 5-hydroxytryptamine that we will keep it here, with the abbreviation 5-HT.

Likewise, 5-hydroxy-indoleacetic acid (abbreviated as 5-HIA acid) would be named hydroxy-5-indoleacetic acid, and 5-hydroxytryptophan (abbreviated as 5-HTP), hydroxy-5-tryptophan.

Since January 1, 1956, the term "oxy" has been used instead of "hydroxy" in German literature (Lembeck, 1956 [149]).

finitive finding by Rapport et al., the most important chemical research was conducted by Erspamer, by Bracco and Curti, and by Udenfriend. The synthesis of 5-hydroxytryptamine was accomplished in 1951, simultaneously and independently by Hamlin and Fisher [114] and by Speeter et al. [241].

(*a*) The formula for 5-HT reveals its close relationship to tryptophan, from which it is derived by oxidation followed by decarboxylation (Titus and Udenfriend, 1953 [249]). In fact, tryptophan is transformed into hydroxytryptophan under the influence of an oxidase present in liver and kidneys, then the oxidated form is subjected to the action of a decarboxylase which is present in the mucous membrane of the gastrointestinal tract and other tissues (lungs, etc.). The amino acid becomes an amine, an indole-alkylamine.

(*b*) Degradation. Free 5-HT is rapidly destroyed by the action of an oxidase present in blood and in numerous organs (lungs, etc.). This oxidative deamination transforms 5-HT into 5-hydroxy-indoleacetic acid. This compound, which is very similar to heteroauxin and well-known to biologists, is detectable in urine. Thus the importance of 5-HT metabolism becomes apparent, and it will be discussed later on.

It should be noted that serotonin exists both in free form, susceptible to the action of monoamine oxidase, and in bound form, not subject to this destruction. For experimental purposes, serotonin is usually provided in the form of serotonin creatinine sulfate, which is its natural state. Experimental quantities are expressed in basic 5-HT weight (the amine actually corresponding to one-half of the molecular weight of the chemical compound).

IDENTIFICATION AND DETERMINATION

Methods of identification and determination are direct or indirect depending on whether they are applied to serotonin itself, or to its metabolites.

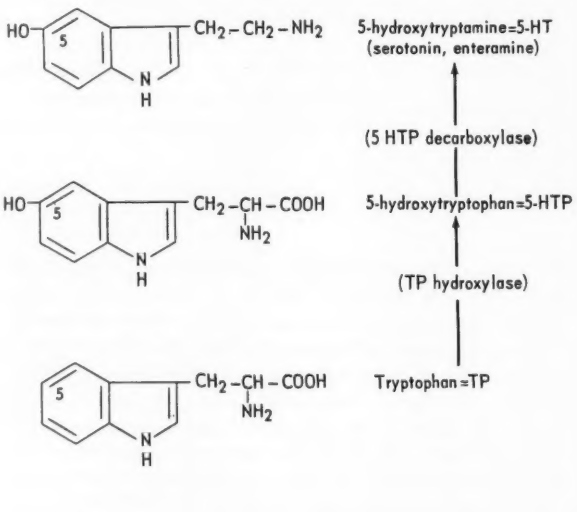
A. *Direct methods.*

Direct methods may be physical (absorption in ultraviolet, colorimetric and fluorometric techniques), chemical (chromatography), or biological. Their sensitivity range is very extensive. Preliminary extraction, optional for organic liquids, must be done with tissues.

1. Physical methods. These have been defined by Erspamer and his collaborators in Italy, by Udenfriend and his collaborators in the United States. In all cases the tissue is homogenized in an acid medium, usually with 2 volumes of 0.1 N HCl.

(*a*) Absorption in ultraviolet. In acid solution maximal absorption is obtained at 275 mμ and another peak at 295 mμ. Concentration should not exceed 0.4 micromole per ml in a unit of the Beckman spectrophotometer of 1 cm depth, otherwise Beer's law would not hold.

TABLE I.—*Biological Synthesis of Serotonin*



(*b*) Colorimetry. This technique is based on the reaction of 5-HT with α-nitroso-β-naphthol diazonium salts giving the violet coloring. In this method, as in the preceding one, the sensitivity is 1μg per ml, which is insufficient for ordinary determinations. But it has the advantage of being suitable for chromatographic analysis.

(*c*) Fluorometry. With a special spectrofluorometer it can be shown that 5-HT and the other 5-hydroxyindoles, when activated at 295 mμ, emit fluorescent light presenting a maximum at 330 mμ. Sensitivity reaches 0.05 μg of 5-HT [257b]. This characteristic seemed insufficient to Udenfriend, Bogdanski, and Weissbach (1955, [157a]), who proceeded with the study of a fluorescence at 550 mμ in acid medium (3 N HCl) after activation at 295 mμ.

2. Chemical methods. Chromatography may be used alone or in association with fluorescence. Several solutions, most frequently with a butyric acid base, have been suggested mainly by Twarog and Page (1953 [254]). These semi-quantitative methods are especially indicated in the assay of synthetic serotonin.

Jepson and Stevens (1953; cf. [154a], p. 37) introduced an elegant solution to the problem of identifying spots on chromatographic paper. After heating the

paper with a mixture of ninhydrin and acetic acid, examination under ultraviolet light shows an intense blue-green fluorescence. Transmission is maximal for a wave-length of 490 mμ, with excitation from a Wood light (365 mμ). This reaction occurs with tryptamines and 5-HT, but not with the N-substituted indoles. It allows identification, but not determination. Jepson believes that the ensuing product is a 1-dihydrocarboline.

3. Biologic methods. These take advantage of the contracture induced by 5-HT on vessels and smooth muscles, which, as we shall see later, is one of the principal properties of this amine. They are useful as semi-quantitative tests.

(*a*) The oldest method uses annular sections of beef carotid (cf. Bigelow, 1953 [20]) or sheep carotid. According to Woolley and Shaw, maximal contraction is obtained with a serotonin concentration of 0.5 to 1 μg per ml.

Half the maximum is given for quantities of 0.08 to 0.2 μg/ml, while sensitivity (liminal contraction) goes down to 0.03 μg/ml.

(*b*) Perfused rabbit ears constitute an excellent reactive according to M. B. Zucker (1955 [284c]), who modified the initial technique of Green and Page (1948). This device is now a classic one. A rabbit ear is removed under ether anesthesia, and the central artery catheterized with a glass cannula, and fed with Ringer solution contained in a Mariotte flask maintained at constant temperature and pressure. The perfusion liquid flows out of the sectioned veins at a rate varying only, all other factors being equal, with the caliber of the perfused system. Each drop falls on a paddle which activates a counter. Experience shows that the same rabbit ear may with care be used as long as 3 to 5 days. Sensitivity varies from one preparation to the other, and in one preparation from one day to the next.

Measuring at each session is based on the principle of dilution of a standard solution of synthetic 5-HT. The sample to be measured is diluted until the standard effect is reproduced. Between measurements, it is satisfactory to clean the vessels with an isotonic saline solution and wait for 5 minutes. Determination of serotonin is also possible by perfusion of a pig's ear (Grette, 1957 [111]) or a guinea pig's ear (Grette, 1957, quoted by J. Levy [151]).

(*c*) Erspamer (1954 [83]) suggested using a rat uterus (virgin female, or better yet, an adult female, ovariectomized several months previously) in estrus induced by injection of estradiol dipropionate 4 to 7 days before the experiment. After preliminary treatment with atropine, the uterine cornu is sensitive to serotonin in 1:200 million dilution (0.5×10^{-5} to 1×10^{-3} μg/ml). Such a preparation reacts only weakly to other known amines and chemical mediators, thereby ensuring a certain specificity. The preparation may be used for 3 to 4 hours. The induced contracture lasts for a while and is measured as a surface.

(*d*) Many researchers now prefer the rat colon, used by Toh and his collaborators (1953). The technique was recently described by D. Quivy (1958 [201]). A 3 cm segment of a rat's terminal colon is placed in Gaddum's survival solution (hypocalcic solution), enriched with oxygen and additional atropine in amounts of 22 τ per liter. Several tests are needed before regular ample contractions are obtained. A solution of known activity is used to take the measurements.

This type of preparation can be used for 35 to 40 responses. J. R. Vane (in [154a], p. 36) cut the small curvature of the rat stomach into narrow strips, and mounted them on a kymographic apparatus, adding hyoscine to neutralize the action of acetylcholine. Leitch et al. (1957 [314]), conducted studies on the sensitivity of the guinea pig ileum and rat uterus to pH variations and atropine content of the perfusion liquid. In acid medium the rat uterus becomes less sensitive to acetylcholine and serotonin, and at the same time more sensitive to atropine.

(*e*) Many other biologic systems are suitable for measuring the contracture induced by 5-HT. One of the most sensitive is the heart of a large mollusk, *Venus mercenaria* (Welsh, 1943); while not sensitive to pitressin and angiotonin, and only mildly sensitive to tyramine, adrenalin, and cinobufotenin, this reactive reveals the presence of 5-HT in concentrations of 0.0018, and even 0.00018 μg/ml.

We will also mention the heart of *Buccinum undatum* (Welsh, 1954 [272]); the sphincter of the sea anemone; the retractor muscle of *Mytilus edulis* (mussel); and in fresh water mollusks, the heart of *Anodonta cygnea L.* (Fänge, 1955 [87]).

We must mention that the results given by these various methods of measurement do not coincide strictly. Chemical methods seem to yield higher figures, according to Udenfriend and Weissbach (1954 [256]). Choice of an assay technique will depend on the sensitivity and specificity desired. Sensitivity was shown for the principal techniques. As for specificity, it can nearly always be secured by the addition of serotonin antagonists, which will be effective when the usual antihistamines and antiadrenalins, for example, are not. The choice of a method will also depend on the equipment of the laboratory, the training of its staff and its geographical location, which may or may not make marine animals easily available.

Spectrophotometry with ultraviolet light is an expensive method; spectrofluorometry is very sensitive (Cartier and Moureau).

Unrelated to determination per se and depending on the nature of the material being studied, the ex-

traction methods are important when, for example, substance P must be eliminated.

Extraction with acetone was criticized by Cargill-Thompson and his collaborators (1958 [51]), who claimed that insignificant values in blood and serum resulted from this method. They suggested that a butanol buffer be added to the heparinized blood, and extraction performed in the butanol phase with a mixture of heptane-0.1 N HCl. Gluckman and Abrams (1959 [306]) described a new chromatographic method by ion exchange, ensuring 100% recovery of 5-HT in homogenates of cerebral tissue. Subsequent determination is effected by spectrofluorophotometry.

The taking of specimens is not unimportant, at least for serum and plasma, since the slightest alteration of the platelets results in premature release of absorbed serotonin; surface problems and choice of anticoagulant are therefore important. Pare et al. (1957 [323a]) noted that during coagulation a fraction of platelet-5-HT passes into the red corpuscles. Therefore they recommend keeping the blood in a waterbath at a temperature of +37° C for an hour without agitation, then collecting the serum by centrifugation.

Sharman and Sullivan (1956 [226]) find a distinct difference in the 5-HT content of serum, depending on whether the blood, previously collected with silicon material, is kept undisturbed or agitated in the presence of glass balls. Higher ratios were obtained with undisturbed blood. When blocking the destruction of 5-HT by ascorbic acid, Davis (1959 [299b]) obtained reproducible, relatively high levels of serotonin concentration in serum. To be free of serotonin, the plasma should come from blood collected unexposed to surface action and without anticoagulants (Cambridge and Holgate, 1958 [50]). The disodic salt of E.D.T.A. (Versène, Séquestrène, Complexon III) prevents lysis and spontaneous agglutination of the platelets, but it acts as a potent decalcifier, and thus may interfere in the response of the isolated organ used for biologic determination.

Heparin, employed alone, brings a release of platelet serotonin in beef plasma (Toh, 1956 [251]).

In human blood and plasma, Waalkes (1959 [267b]) precipitates proteins and extracts 5-HT from a basic solution with ether or N-butanol. After precipitation, determination was made by fluorometry.

B. *Indirect methods*

These consist essentially in the investigation and determination of a serotonin metabolite, 5-hydroxy-indoleacetic acid (note 2), eliminated in urine.

This acid forms a red compound with nitric acid and α-nitroso-β-naphthol. Before determination, it is necessary to eliminate interfering substances and extract with ether (reaction of Sjoerdsma and Udenfriend).

An alternative is to treat with 0.5% of dinitro-2, 4-phenyl-hydrazine (DNP) in HCl 2 N; extract with chloroform; eliminate hydrazine with an ether lavage; use a phosphate solution buffer, and color with a mixture of nitrosonaphthol, buffer solution and nitric acid (technique of Udenfriend, Titus and Weissbach, 1955 [257c]).

Results are read in the spectrophotometer: two maximal absorptions are investigated at 650 mμ (valid for a concentration of 0 to 100 μg/ml) and at 525 mμ (concentration of 0 to 15 μg/ml). The second maximum may be investigated at 540 mμ (DeGennes et al.).

These techniques have been adapted in various ways: qualitative and quantitative applications exist (cf. DeGennes et al., 1957 [72], Cartier and Moureau, 1958 [55], and Pierce, 1958 [193]).

Colorimetric determination may be preceded by chromatography or paper electrophoresis. Carcasona et al. recently presented a spectrophotometric method (Klin. Wchnschr. 1959, 37: 253-254), and a chromatographic method (id. 761-763) for the determination of 5-HT and 5-HIA acid.

However, 5-HIA acid is not the only compound which can originate from 5-HT: through a new method of indole extraction, McIsaac and Page (1958 [160a]) have identified the following substances in the urine of patients suffering from carcinoid tumors: -hydroxy-5-indoleaceturic acid, and -N-acetyl-hydroxy-5-tryptamine.

In practical use, the marked quantity of 5-HT in banana pulp (Lewis, 1958 [153]) might lead to errors in the determination of urinary 5-HIA acid in subjects having eaten bananas. Actually, conversion of this alimentary serotonin in the organism is rapid and complete, since renal excretion of oxidation products is effective within 8 hours. Diet should nevertheless be taken into account when interpreting the results.

CHAPTER II

Distribution in Nature and in the Organism

Distribution in nature. 5-HT is widely distributed in nature. We can examine this distribution as does Collier (1957 [59]): (*a*) in living species; (*b*) in organs and tissues; (*c*) in cell types; (*d*) within the cell itself.

A. DISTRIBUTION IN LIVING SPECIES

(*a*) Animal kingdom. The presence of 5-HT has been shown in all vertebrates, principally in the intestine, but also in the venom of certain amphibians such as the toad, and in the digestive tract of low forms of animal life (*Tunicata*). It was detected by Fantl and Ward (1957 [88]) in the serum of marsupials. In mollusks, it is found in the posterior salivary glands of *Octopus vulgaris* and of *Eledone moschata;* in the nerve ganglions of *Venus mercenaria* (Welsh, 1954), and the byssus retractor of the mussel *Mytilus edulis* (Twarog, 1954); in the hypobranchial corpus of *Murex trunculus* (Erspamer). 5-HT was also discovered in the venom of several insects, such as the wasp, and certain spiders (scorpion). At the bottom of the animal scale, only the more developed Coelenterata (sea anemone and physalia) seem to possess 5-HT in their nematocysts (Welsh, 1955). However, this does not allow us to infer with certainty the meaning of the appearance of 5-HT in evolutionary phenomena.

(*b*) Vegetable kingdom. Since tryptophan is generally distributed throughout all living species, its direct chemical kinship with 5-HT, emphasized by Niaussat et al. (1958 [178]), establishes a bridge between animal and vegetable kingdoms. In man, oral administration of 3-indolyl-acetic acid is followed by increased elimination of 5-HIA acid (Rysanek and Vitek, 1959 [328]). The general role played by indolic compounds in vegetable growth has been known since Salisbury. But the correlation goes further; 5-HT itself is present in the spicules of the stem of a dicotyledon *(Mucuna pruriens),* and in the hairs of the white nettle *(Urtica dioica)*; this fact was established by Collier and Chesher, using an elegant technique, on the imprint of a nettle leaf. Recently 5-HT was detected by chromatography in higher plants, in the pericarp of young fruits of *Gossypium hirsutum,* and in the leaves of an Aracea, *Symplocarpus foetidus* (Bullard and Car, 1958 [294]). On this occasion the authors remind us that 5-hydroxy-indole derivatives have been determined in a wide variety of species. In its natural state 5-HT is present in banana pulp in relatively high concentrations: 28 μg per gram (Anderson et al., 1958; Waalkes et al., 1958, quoted by Lewis, 1958 [153]).

B. DISTRIBUTION IN ORGANS AND ORGANIC TISSUES OR LIQUIDS

This distribution has been studied very carefully, especially in man, dog, and rat, and was the subject of detailed classification (Collier [59], Twarog and Page [254], etc.) It is far from uniform and shows a hierarchy which puts the digestive tract in first place. Erspamer (1954 [83]), Feldberg and Toh [89] found the following values respectively for stomach, small and large intestines: 5.2, 4.0, and 2.8 μg/g (dog), and 1.4, 1.2, and 3.9 μg/g (rat). Feldberg and Toh found up to 8.5 μg/g in the duodenum of the dog; this value is exceeded only in some pathologic tissues, as we shall see later on. In the dog, 80% of the 5-HT contained in the whole organism is found in the intestinal mucosa, almost exclusively in the mucous membrane; submucosa, muscularis mucosa, and external muscle contain almost none. The spleen is rich in 5-HT: 1.4 to 4.6 μg/g—figures vary with authors—is found in the dog; 2.5 to 2.8 in the rat.

Dog liver, and rat lungs and skin contain significant quantities of 5-HT; respectively: 0.54, 1.2, and 1.34 μg/g (Weissbach, Waalkes, and Udenfriend [269]).

It was Page who discovered that 5-HT was always present in nervous tissue: 0.1 to 0.36 μg/g in dog, rat, and rabbit (Twarog and Page, 1953); its distribution there varies greatly at different levels. The distribution of 5-HT in the cerebral tissue of the dog (essentially in the gray matter), is very similar to that of noradrenalin (Amin et al., 1954 [2]). The placenta is poor in 5-HT (0.13 ± 0.04 μg/g) (Kuriaki and Inoue, 1956 [142]). 5-HT content varies greatly in the principal organic fluids: the cerebrospinal fluid is practically devoid of it. So is plasma without platelets; as a matter of fact, the absence of serotonin in plasma, as opposed to its presence in serum, initiated the search for its localization in blood platelets.[3]

Following are values expressed per ml [in μg] for whole blood:

Man	*Guinea Pig*	*Rat*	*Dog*	*Cattle*	*Rabbit*
0.16 + 0.06 [1]	0.15 [2]	0.20 [2]	0.20 [2]	1.70 [2]	4.0 [2]
0.22 [2]	0.30 [2]	0.30 [2]	0.25 [2]		4.5 [2]
0.10 [3]					
0.30 [2]					

[1] Hardesty and Stacey, 1955 [115].
[2] Udenfriend et al., 1957 [259].
[3] Udenfriend and Weissbach, 1954 [256].

Serotonin content of human blood serum is close to 0.1 μg/ml; it varies with methods of specimen taking and of determination:

Serotonin content, in μg (investigator)	*Specimen*
0.62 ± 0.028 (0.03 to 0.13) (Zucker)	perfused rat ear
0.12 (0.07 to 0.20) (Erspamer)	rat uterus
0.07 ± 0.02 (Snow)	acetone extract—rat uterus
0.098 (0.044 to 0.247) (Barkhan, 1955 [12])	rat uterus
0.09 to 0.18 (Basserga and Ballerini, 1956 [14])	colorimetry
0.154 and 0.160 * (Sharman and Sullivan, 1956 [226])	rat uterus
0.0456 ± 0.0058 (Quivy, 1958 [201])	terminal rat colon
0.21 (0.10 to 0.32) (Davis, 1959 [229b])	spectrofluorometry

* But when the tube containing blood was agitated, the content was 0.075 and 0.116 μg.

[3] We fail to understand Inoue and Kuriaki's figures (1957 [31]). They give the 5-HT content of citrated guinea pig plasma as 0.1 to 0.14 μg per ml.

In dog serum: 0.08 μg (Erspamer); 0.57 μg (Twarog and Page, Erspamer). In rat serum: 0.57 to 1.73 μg (Erspamer, 1954). Urine contains appreciable quantities of serontonin: between 0.1 and 1 μg/ml, in human and dog urine. (Twarog and Page). Kuriaki and Inoue (1956 [142]) measured serotonin in the urine of pregnant women, and found that values were 3 to 4 times higher than in controls (between 0.56 to 0.95 μg compared to 0.23 μg in controls). Amniotic fluid does not contain 5-HT, according to Erspamer (1954 [83]).

Variations with Age

According to Faustini, quoted by Mitchell and Cass (1959 [310]), it seems that serotonin is present in the organism even during uterine life, at least in the animals that were studied. Mitchell and Cass found a very slight quantity of 5-HT at birth, determined in the blood of the umbilical cord. This anomaly, according to the authors, is due to the inability of the infant to fix serotonin in the platelets, not to a defect in serotonin production (5-HT is found in the intestine of the fetus). This ability suddenly appears between 4 and 5 months; from the age of 20 weeks on, and without appreciable variation in the number of platelets, the 5-HT content of whole blood and of separated platelets is comparable to that of an adult. In healthy children of 1 to 12 years, the authors found 0.124 μg per ml of blood, and 0.423 μg of 5-HT per billion of platelets.

Pare et al. (1957), quoted by the above mentioned authors, found that the 5-HT content of serum during late childhood was 0.124 μg/ml (mean of 15 determinations).

C. DISTRIBUTION IN CELL TYPES (NORMAL OR PATHOLOGIC CELLS)

We shall consider three types of cells or elements: (*a*) argentaffine cells; (*b*) blood platelets; and (*c*) mast cells.

(*a*) With argentaffine or enterochromaffine cells, sometimes called Nicolas Kultschitzky cells, we come to the principal source of serotonin.

In the Kultschitzky cells of the duodenal mucosa (Lieberkühn crypts) Gosset and Masson, as early as 1914, had revealed granules that reacted with ammoniated silver nitrate. They found the same intracellular granules again in carcinoid tumors of the appendix; this was confirmed much later by Pearse, according to Peskin and Orloff (1959 [191]).

In these same cells Erspamer had localized the origin of the substance he called enteramine, long before chemical identification was possible.

Under the name of enterochromaffine, Erspamer designated "cells whose granules show chromaffin and argentaffin reactions, coupled with diazonium salts and fluoresce after fixation with formaldehyde." Collier, from whom we are borrowing this definition, disputes whether it is well-founded. The histochemical reactions mentioned above are not absolutely specific. 5-HT can be found in cells not presenting these characteristics. Conversely, cells of the intestinal mucosa of teleostei answering this histochemical definition do not produce 5-HT; for this reason Erspamer calls them pre-enterochromaffine. In mammals, the autonomy of the enterochromaffine system, comprising the enterochromaffine cells of the gastro-intestinal mucosa, bile ducts, and principal pancreatic ducts, is none the less well established.

Disputable is the concept expressed in Page's thesis (Montpellier, 1955 [188]). He includes the enterochromaffine system in a broad whole, the clear cell or "peripheral endocrine system" of Feyrter (1931-1938).

If one shares this viewpoint, the origin of 5-HT might be more diffuse than assumed by Erspamer, and the amine might originate outside the gastrointestinal tract.

Let us remember that in fact the largest quantity of 5-HT was shown in the gastrointestinal mucosa in vertebrates and *Tunicata* (up to 80% of the total organism in the rat).

On the other hand, Toh demonstrated (1954 [250]) that specimens of whole blood from the portal vein are much richer in 5-HT than specimens of femoral arterial blood. The perfused dog stomach spontaneously releases 0.05 to 0.5 μg, and exceptionally 0.8 μg of serotonin per minute.

Rosenberg et al (1959 [326]) studied cases of massive resection of the gastrointestinal tract in man and animal; only after total resection do serum levels of serotonin fall, as well as urinary secretion of 5-HIA. 5-HT secretion at physiologic level is assured as long as 5 cm of the terminal duodenum and first part of the jejunum are preserved.

(*b*) Presence of serotonin in blood platelets. Serotonin is present in whole blood and in serum, but not in plasma collected by vigorous centrifugation. The 5-HT content of whole blood and serum is therefore revealed by the blood corpuscles, specifically by the platelets.

The figures below [in μg] are given per billion platelets (some authors prefer to estimate per azote weights: [4]

[4] In human platelets, our own estimates give 1 billion platelets = 0.31 mg azote.

Man	Rat, Guinea Pig	Cat	Dog	Pig	Goat	Rabbit
0.2 to 0.3 [1]	0.2 to 0.4 [1]	0.9 [1]	1.7 (0.6 to 2.7) [7]	2.2 [1]	4.3 [1]	7.3 (4.5 to 12) [1]
0.06 ± 0.02 [2]						
0.5 to 1.3 [3]						
0.57 ± 0.17 [4]						
0.87 (0.5 to 1.5) [5]						
0.52 and 1.21 ± 0.23 [6]						

[1] Humphrey and Jacques, 1954 [130b].
[2] Snow, 1955.
[3] Zucker and Borrelli, 1955, using perfused rabbit ear.
[4] Hardisty and Stacey, 1955 [115].
[5] Weiner and Udenfriend, 1957 [275], using classic methods.
[6] Polli et al., 1958 [197], using spectrofluorometry.
[7] Humphrey and Toh, using atropinized rat colon.

Direct correlation between the serotonin content of whole blood and its richness in platelets was confirmed by Kerby and Taylor (1959 [311]), measured by fluorometry.

In normal man, Polli et al. considered the influence of age and found in subjects over 70 0.82±0.22 μg of 5-HT per billion platelets, compared to 1.21 in controls.

We will remember here that, according to Mitchell and Cass, the platelets of newborns are practically devoid of serotonin; they become charged with 5-HT only between the 16th and 20th weeks.

The other blood cells, notably the polynuclears, are completely devoid of 5-HT.

We shall return later to the relations between blood platelets and serotonin: let us just remember that the platelets constitute a "circulating reservoir" of 5-HT.

(c) Presence of 5-HT in mast cells has been considered following three main observations:

1) direct demonstration by Benditt et al. (1955 [18b]) of serotonin in mast cells isolated from the irrigation fluid of rat peritoneum (Giarman and Potter, 1958 [106]);

2) liberation of pharmacologically active substances from a perfused rat leg, caused by histamine-displacing substances (Lewis et al., 1956; Rowley and Benditt, 1956);

3) extraction of 5-HT from rat skin, and reduction of its level with 48/80 which destroys mast cells (Parratt and West, 1957 [189a, b]).

Finally, the areas of rat skin which are richest in mast cells are also richest in histamine and in 5-HT.

Consequently, all these authors tend to ascribe the relatively high content of the rat skin in 5-HT, if not in histamine, to the mast cells alone. But the phenomenon is not general as in the platelets; according to Lewis, the mast cells of cat and dog contain histamine, but little or no 5-HT.

Variations in animal species are considerable (Sjoerdsma et al., 1957 [236]).

Ende and Cherniss (1958 [82]) see no correlation between the abundance of mast cells and serotonin content in the spleen.[5]

[5] We do not find this objection very valid, for the spleen is far richer in platelets than in mast cells.

On the other hand, serotonin is not found in all mastocytoma (Parratt and West, 1957). Furth et al. (1958 [96]), however, identified through chromatography a large amount of 5-HT (140 μg/g), as well as histamine and heparine in a transplantable mastocytoma of the mouse.

Giarman and Potter (1958 [106]) also mention the high serotonin content of a mastocytoma in the mouse.

Here again different species vary: mastocytomas in dog or man contain no 5-HT.

In mastocytomas of the dog, Meier (1959 [318]) was able to reveal only slight amounts of serotonin by the technique of Udenfriend et al. (1955) while mastocytes of the mouse and rat contained large amounts (Cass et al., 1958 quoted by Meier).

Ono et al. (1959 [322]) in discussing the high content of 5-HT, histamine, and heparine in the transmissible mastocytoma of the mouse, advanced the following theory: the mastocytes would represent an independent type of cells, capable of synthetizing and storing the three substances in their specific granules. 5-HT was determined by action on the heart of a mollusk.

D. LOCALIZATION IN THE CELL ITSELF

Apparently, cytoplasmic granules, stainable with silver or chrome, are the vehicle of 5-HT formed in the enterochromaffine cells. Blaschko was able to isolate these granules by differential centrifugation of intestinal mucosa of dog, after homogenization (1957 [25]).

The granules of tumor cells (carcinoid) have the same histochemical character as Kultschitzky cells (Pearse, quoted by Peskin and Orloff [191]).

The sites of 5-HT fixation by the platelets may be those of ATP-localization. Born et al. demonstrated the affinity of ATP for serotonin (1956 [29]).

CHAPTER III

Metabolism of 5-Hydroxytryptamine

The principal methods of synthesis in vivo and of degradation of 5-HT are now well known. Weissbach devoted his Ph.D. thesis to this subject (1957 [268]), and recently Donaldson and Gray (1959 [300b]) presented an excellent review.

A. SYNTHESIS IN VIVO

The biologic synthesis of serotonin has been studied in man, toad, and bacteria (Udenfriend et al., 1956 [258b]). The starting point of 5-HT, and generally of all indolealkylamines, is tryptophan, an amino acid widely distributed in nature (Udenfriend, Clark and Titus, 1953). Synthesis is achieved in two stages, oxidation and decarboxylation, each of which is catalyzed by a specific enzyme system; there exists therefore a tryptophan oxidase and a 5-hydroxytryptophan-decarboxylase. By localizing these enzymes, it is possible to find the sites of origin of 5-HT. Their distribution in the organism is somewhat surprising (Gaddum and Giarman [97]).

First stage: Oxidation of tryptophan. Tryptophan plays an important role in general metabolism; only a small fraction of this amino acid is transformed into 5-hydroxytryptophan. This fraction is considerably increased (up to 60%) in subjects with carcinoid tumors as well as in mice with certain mast cell tumors. Udenfriend and his collaborators were the first to observe the conversion of tryptophan into its oxidative derivative in the cutaneous glands of the toad. Tryptophan oxidase (also called hydroxylase), an enzyme, well defined by its substrate, is found in large amounts in the liver and kidneys of mammals.

Two isomers exist, levo and dextro, of 5-OH-tryptophan: only the levo form, specifically dietary tryptophan, is susceptible to the enzymatic action of decarboxylase (Udenfriend).

Second stage: Decarboxylation of 5-OH-tryptophan. The following method is used for the determination of 5-OH-tryptophan-decarboxylase: tissue homogenates are incubated with 5-OH-tryptophan in the presence of p-tolyl-oxy-choline ether, an amino oxidase inhibitor. The resulting 5-HT is measured (Giarman, 1956 [105]). Gaddum and Giarman (1956 [97]) showed with this method that 5-OH-tryptophan-decarboxylase abounds in the kidney, liver, and digestive tract; that it is also present in nervous tissue (sympathetic ganglions and sometimes central nervous system); but not present in bone marrow, in plasma rich in platelets, or spleen.

Decarboxylase is at an optimum at pH 8.1. Its action requires a co-enzyme, pyridoxal phosphate. Pyridoxine-deficient chickens have a low serotonin content in blood and tissues. Serotonin is a "biogenic amine", like tyramine, dopamine, noradrenalin and histamine.

The similarity can be underlined in the method of synthesis as follows: 5-HT derives from 5-HTP by decarboxylation, just as dopamine or hydroxytyramine derives from 3-4-di-hydrophenylalanine, or DOPA (Holtz, 1958 [309]). 5-HTP-decarboxylase may be identical with DOPA-decarboxylase (Westermann et al., quoted by Holtz): both enzymes have the same localization and are sensitive to pyridoxal-5-phosphate.

Vitamin B-6 deficiency in the chicken causes a reduction in the 5-HT content of various organs (Fox et al., 1955, quoted by Holtz); in the rat the reduction is particularly marked in the spleen tissue and coincides with a reduction of the 5-HTP-decarboxylase content of the kidney (Yeh et al., 1959 [338]). In the deficient rat, adrenalectomy increases and cortisone decreases the 5-HT content of liver tissue.

These indications are extremely interesting. If decarboxylation is the last stage leading to 5-HT, the latter can only originate in organs or tissues that contain decarboxylase as well as the substrate. The digestive tract and nervous system have a claim to 5-HT synthesis, while platelets are unlikely to produce the serotonin they are transporting.

In the nervous system, we know that 5-OH-tryptophan is able to cross the blood-brain barrier, while 5-HT injected into a vein does not reach the brain. Udenfriend et al. (1957) noted that 5-OH-tryptophan administered to animals, is retained in tissues containing specific decarboxylase, and rapidly transformed into 5-HT (which may reach up to 10 times its normal rate in the blood). Bogdanski, Weissbach and Udenfriend (1956 [26]) were able to reproduce the predominant nervous effects of 5-HT by injecting 60 mg/kg of 5-hydroxytryptophan into the veins of a dog.

Administration of 5-OH-tryptophan to the rat, continued for 4 months, markedly raised serotonin levels of the brain, kidney and liver, at the same time causing diarrhea and vaso-motor disorders of the skin (Davidson et al., 1957 [70]).

In man, continued administration of this precursor is followed by a lasting increase of serotonin in the tissues, with stimulation of the gastro-intestinal tract (id.).

5-OH-tryptophan raises the serotonin content of the brain in normal rabbits, as long as they are not treated with reserpine (Kunzman et al., 1956 [141]).

In rabbits receiving 5-OH-tryptophan, not only does serotonin increase in its bound sites, but it appears in tissues such as liver, kidney, heart, and uterus (Udenfriend et al., 1957 [261b]).

Bogdanski et al. noted a strict parallelism between the distribution of 5-HT and of decarboxylase in diverse sections of the dog encephalon.

Repeated administration of reserpine to dog and rat has practically no effect on the biosynthesis of 5-HT (Erspamer and Ciceri, 1957 [85]); the metabolism of the drug is deviated, but nevertheless remains important, as evidenced by the excretion of 5-HIA acid. We know that reserpine does not act on decarboxylase (Kuntzman et al., 1956 [141]).

Finally, it must be remembered that isotope techniques have provided unquestionable confirmation of

the biologic origin of serotonin; Udenfriend and Weissbach (1954 [256]), after injecting rats with tryptophan tagged with an atom of carbon, were able to localize an action on platelet serotonin within the next few hours.

The 5-HT content of brain and intestines is progressively and markedly decreased in rabbits, guinea pigs, and mice subjected to a tryptophan-deficient regimen. In severe deficiency, histochemic reactions of the enterochromaffine cells are also diminished (Zbinden, Pletscher and Studer, 1958 [278b]).

Is integrity of the liver necessary for 5-HT synthesis? The data collected on this subect are somewhat contradictory; according to Borges, Merlis and Bessman (1959 [27c]), patients suffering from severe hepatic insufficiency are still able to metabolize 5-HTP in the same degree as healthy individuals of the control group. According to Donaldson et al. (1959 [300]), chronic cirrhotic patients synthetize less 5-HT than healthy subjects or subjects suffering from extrahepatic biliary obstruction. 5-HT metabolism seemed to be reduced in chronic alcoholics, according to Gursey et al. (1959 [307]). In the rabbit, repeated intravenous injection of ethanol decreases the 5-HT content of brain tissues.

In addition to the fundamental chemical research done on in vitro synthesis by Hamlin and Fisher and by Speeter, we should remember the following fact: Doepener and Cerletti (1958 [79]), succeeded in transforming 5-OH-tryptophan into 5-HT in vitro, under the sole influence of ultraviolet irradiation; the resulting transformation is far more important than that of histamine into histidine under the same conditions. Biologic in vitro synthesis has been achieved many times with tissue extracts to which a known quantity of 5-hydroxytryptophan had been added. Transformation into 5-HT confirms the presence of the enzyme responsible for decarboxylation (cf. Gaddum and Giarman, 1956 [97]). Biosynthesis of 5-HT with intestinal bacteria possessing several carboxylases is possible, but has not been demonstrated (Holtz, 1958 [309]). Abundance of decarboxylase in kidneys and intestines, and dearth in muscular tissues, has been confirmed by perfusion results of the hind part or of isolated organs of the rat: 1 mg of 5-HTP plus pyridoxal phosphate is injected by arterial cannula, and 5-HT is determined in the effluent (Sandler and West, 1958 [220a]). 5-HT output is not modified by preliminary treatment with polymyxin-B (which releases tissular histamine); it is produced early, but not increased by iproniazid; it is practically doubled by preliminary reserpine treatment.

B. BIOLOGIC FATE

A small portion of circulating 5-HT is eliminated in urine without being modified. Blaschko [25] believes that the concentration of produced serotonin is diminished by diffusion through surrounding fluids, or by enzymatic inactivation. We are particularly interested in enzymatic degradation. 5-HT degradation has been studied in vivo in numerous organisms. In vitro it was achieved with fresh tissue extracts, and with bacteria. Some soil bacteria can use 5-HT as their only source of carbon, when adapted by preliminary culture in the presence of this amine. The oxidative method is used, but oxidation exceeds 5-HIA-acid, and the acid itself also undergoes marked degradation (Oginsky et al., 1958 [321]).

5-HT degradation occurs in several ways. The main one—at least in man—and best known is the transformation into 5-OH-indoleacetic acid. Since this acid is eliminated in urine, its determination in urine allows comparison of 5-HT metabolism in normal and pathologic conditions.

When 21 mg of 5-HT are injected into the veins of a dog, 40% of the amine are found in the urine under the form of 5-HIA acid within 24 hours; maximal elimination takes place about the fourth hour (Titus and Udenfriend, 1954 [249]). 5-OH-indoleacetaldehyde, a hydroxyindole, seems to be the intermediary, as demonstrated by blockage with a semi-carbazide (Titus and Udenfriend, 1954). The transformation corresponds to oxidative deamination, catalysed by an amino-oxidase; this action was demonstrated in vitro (Freyburger, 1952; Blaschko, 1952) and in vivo (Erspamer).

Weissbach recently succeeded in isolating the enzyme in soluble form, by completely separating it from aldehyde-dehydrogenase activity, with which it is usually associated in natural products.

An interesting process in the study of 5-HT degradation is the elective monoamine oxidase inhibition by iproniazid (Marsilid l-isonicotinyl-2-isopropyl-hydrazid). Serotonin accumulation in the organism is artificially demonstrated this way (experimental hyperserotoninemia: cf. Kabakow et al., 1958 [136]), specifically at the level of the central nervous system. Iproniazid administration regularly increases 5-HT content of the platelets in man and rabbit, up to 135% on the tenth day (Pletscher and Bernstein, 1958 [196b]). Isoniazid action is less clear.

A new inhibitor of 5-HT catabolism, active in vitro and in vivo in rat brain, was described by Maass and Nimmo (1959 [316]); this substance is product SKF-385, or 2-phenyl-cyclopropylamine. Another MAO-inhibitor is beta-phenyl-ethyl-hydrazine (cf. Dubnick et al., 1959 [301]).

Where can monoamine oxidase be found? Principally in the liver, where it may act as a regulator of amines formed by intestinal bacteria, and entering through the portal vein. Another theory is that MAO might counteract inundation of the general circula-

tion with free 5-HT. MAO is also found in brain tissue and in the lungs.

MAO distribution in the animal kingdom has been extensively discussed by Blaschko (1957 [25]).

Are we confronted with a unique diastasis, or are there several forms of MAO? This question has been debated but remains unsettled. On the other hand, inactivation of 5-HT cannot be the only action of MAO. Conceivably, it may inactivate other catecholamines, such as adrenalin and noradrenalin (cf. Gordon et al., 1958 [109]), dopamine, beta-phenylethylamine, but this older concept calls for confirmation. In vitro, dopamine and epinine constitute an important substrate for MAO. But oxidation leading to formation of 5-HIA acid is not the only degradation process of 5-HT. Erspamer indicated in 1955 that this acid represented only a fraction of the administered serotonin, 33% in the rat, 20% in man, and 1% in the rabbit. In vitro, the liver and kidneys of the rat convert less than 30% of 5-HT into 5-HIA acid. A portion of 5-OH-acetaldehyde may escape transformation into 5-HIA acid to form pigmented and highly fluorescent substances (Blaschko, 1952).

Chadwick and Wilkinson (1958 [298]) believed they could identify, provisionally at least, the brown pigment appearing in rat liver homogenates with O-sulfate of 5-HT. The same substance could be found in the urine of persons suffering from carcinoid tumors (Dalgliesh).

Another method of inactivation was suggested by Porter et al. (1957 [198]), and by Nakajima and Thuillier (1958 [177]): oxidation induced with ceruloplasmin, the globulin-copper complex present in Cohn's fraction IV-1, which has the characteristics of an oxidase. The reaction results in a p.-quinone-imine derivative.

McIsaac and Page (1958 [160a]) consider other methods of degradation: N-acetylation, giving N-acetyl-5-HT; conjugation with glycine, forming 5-hydroxyindoleaceturic acid; conjugation with glucuronic acid, resulting in a glucuronide of serotonin.[6]

Recently McIsaac and Page (1959 [160b]) were able to follow the course of beta-^{14}C-labeled 5-HT in rabbit and rat: they measured activity in various tissues, urines, and feces. They defined the nature of the metabolites encountered in urine through each investigation method: chromatography, radio-autography, fluorescent spectrum, biologic activity. Following are their results: 35% to 83% of exogenous serotonin were metabolized by oxidative desamination—5 to 25% by N.-acetylation—the rest (5 to 10%) resulted in minor metabolites, one of which was provisionally identified as an oxidation product.

The urine of rats supplied a mixture of 5-HIA acid and 5-hydroxyindoleaceturic acid; the urine of rabbits, the glucuronic constituent. Thereby McIsaac and Page confirmed the difference between species previously noted by Erspamer: 5-HIA acid constitutes the major metabolite of 5-HT in carnivores; it is practically non-existent in herbivores. Normal man eliminates daily 2 to 8 mg of 5-HIA acid (Udenfriend et al., 1955 [257c]); this quantity is not appreciably modified in hypertensives (Poumailloux and Labignette, 1959 [199a]). In pregnant women, massive reduction in urinary elimination of 5-HIA acid is notable from the third month of pregnancy; levels decrease from 4.4 gamma to 0.6 gamma/ml, then rapidly increase again after childbirth (Bettendorf et al., Klin. Wchnschr., 1959, p. 936 [37]). In human pathology, major variations occur in the carcinoid syndrome. In man suffering from carcinoid tumor, the metabolic fate of exogenous serotonin is comparable to its fate in the rat.

Monoamine oxidase has been reported to regulate the action of endogenous serotonin as cholinesterase does the action of acetyl-choline (Udenfriend et al., 1957). The complexity of 5-HT inactivation mechanisms greatly limits this assumption. In vitro, 5-HT easily forms bufotenine.

C. IMPORTANCE OF 5-HT-METABOLISM

Our knowledge of precursors and metabolites has made it possible to evaluate the endogenous metabolism of 5-HT. According to Erspamer, the dog and rat completely renew their store of 5-HT in 12 to 48 hours. Daily elimination of 5-HIA acid in urine alone reaches 2 to 3 mg in the dog, 10 mg in man. These values indicate that 5-HT metabolism in the organism is far more important than was initially believed (Titus and Udenfriend, 1954 [249]).

Certain points were clearly defined by the use of radio-isotopes: by administration of C^{14}-tryptophan, Udenfriend and Weissbach succeeded in identifying the preferred sites of 5-HT formation, and in calculating the turnover of the substance.

The half life of 5-HT was estimated by these authors at 1 or 2 hours in the brain of the rabbit, 17 hours in its intestine, 10 hours in the stomach, 33 hours in the platelets and spleen.

Table II shows various possible fates for 5-HT.

Is it possible to speak of regulation of serotonin levels in the organism? Following are arguments in favor of endocrine participation:

According to Hicks and West (1958 [308]), cortisone and hydrocortisone in daily doses of 50 mg, applied for 14 days, reduce the 5-HT and histamine con-

[6] Weissbach, Redfield, and Udenfriend (1958 [271]) have purified and identified this compound. Its hydrolysis by a beta glucuronidase gives equal amounts of 5-HT and glucuronide. The authors consider this metabolite important (up to 25% of the serotonin injected into the mouse).

tent of rat skin by approximately 50%. The authors believe that cortisone, and glucocorticoids in general, decrease decarboxylase activity, while mineralocorticoids are inactive. Experimental hyperthyroidism in the rat is accompanied by a significant increase of cerebral serotonin levels, also observed in hypothyroidism, but not after thyroidectomy (Towne, Put, and Schwartz, 1959 [334]). These discrepancies may be explained by a direct action on the central nervous system of the propyl thiouracil given in order to induce hypothyroidism.

CHAPTER IV

Pharmacology of 5-HT

In 1957, Udenfriend et al. write [259]: "Only a few years have elapsed since the isolation and identification of serotonin. However, the intense interest in this compound has advanced our knowledge of its biochemistry and pharmacology to such an extent that the information may even help in studies on some of the older agents such as histamine, epinephrine, and norepinephrine. That serotonin is an important agent can no longer be questioned. It remains now to determine exactly where and how it functions."

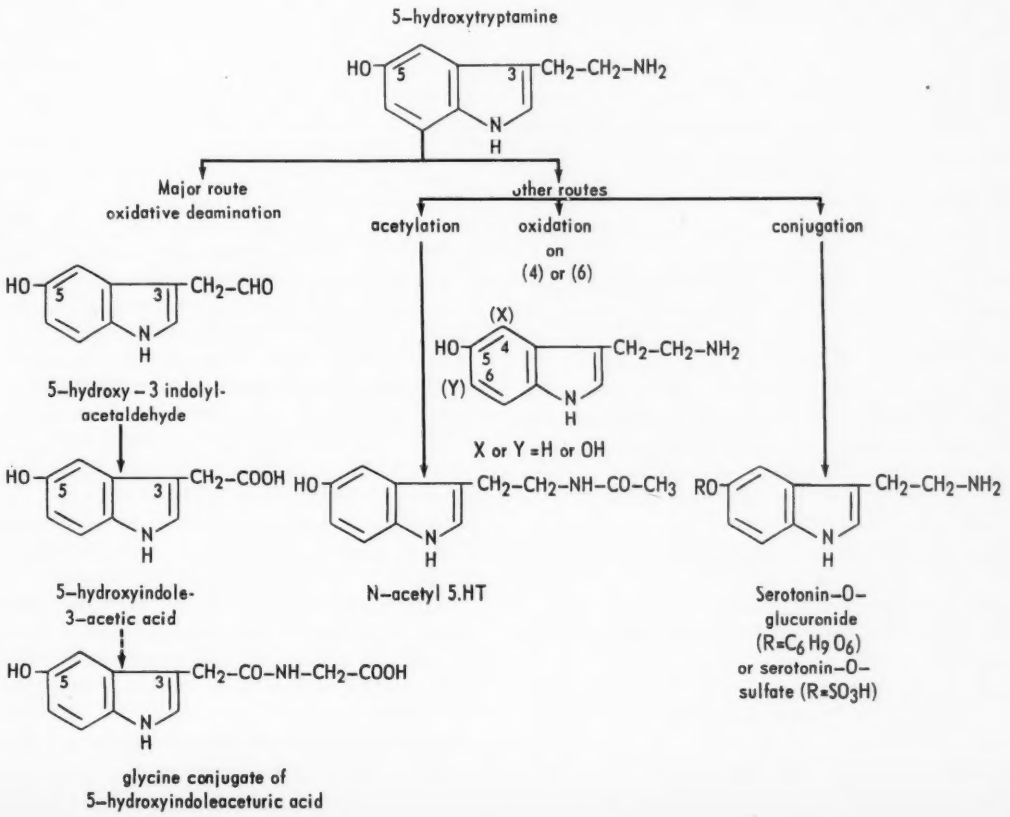
Two years later, the problem has not been solved, or rather, there still are a great number of problems.

In the pharmacologic study of 5-HT, we should go from the simple to the complex by first investigating elementary actions on smooth muscles, isolated vessels and organs, then the more general actions on the cardiovascular system, and finally the broad functions, such as regulation of blood pressure, respiration, diuresis, and hemostasis. We already mentioned that we would exclude the action on the nervous system from this report. However, multiple interferences, linked to the sum of material we are trying to analyze, will compel us to depart from this plan.

A. ACTION ON SMOOTH MUSCLES

Though 5-HT exerts an action on striated muscles (cf. J. Levy, 1957 [151]), its action on smooth muscles

TABLE II.—*Fate of Serotonin in the Organism (partly according to McIssac and Page, 1959 [160])*



is the most characteristic. We have already noted the extensive use of isolated vascular sections, perfused rabbit ear, rat uterus, and guinea pig ileum, for the determination and identification of serotonin. These preparations enabled us to analyze the conditions under which 5-HT induces contracture. The effect is not inhibited by atropine (which suppresses only the cholinergic component in the guinea pig ileum and rat uterus [151]) nor by hyoscine, methonium, or antihistamines such as promethazine. Nicotinamide in high doses is an antagonist of 5-HT action on smooth muscles in vivo and in vitro (Woolley, 1958 [277b]). These properties clearly differentiate 5-HT from acetylcholine and histamine. On the other hand, serotonin-induced contraction is definitely suppressed by LSD-25, BOL-148, and other anti-metabolites (BAS), which therefore are called anti-serotonins. To these we shall devote a special chapter. The use of serotonin as an oxytocic has been considered in clinical obstetrics (Valseghi, 1948 [337]). Mollusk hearts have proved far more sensitive to serotonin than to adrenalin or eserine. 5-HT action on smooth fibers accounts for its in vivo effect on the digestive tract and the bronchi. It does not equally well explain its action on the cardiovascular system.

B. ACTION ON THE CARDIOVASCULAR SYSTEM

Serotonin came to the attention of physiologists by its vasoconstrictive properties in vitro. We must admit, however, that the cardiovascular effects of 5-HT are complex, that they vary considerably with animal species and experimental conditions, and are even now given a diversity of evaluations. The material presented here is taken from reviews by Page (1954 [183], 1958 [187b]), Erspamer (1956), J. Levy (1958 [151]), and from more recent literature.

Effects on Blood Pressure

(*a*) In the dog, according to Page (1954 [183]), the typical response is biphasic; intravenous injection of 5-HT (0.06 to 0.12 mg) into a dog under phenobarbital anesthesia causes temporary hypotension with bradycardia, followed by a rise in blood pressure lasting about 10 minutes. Sometimes another fall in pressure is observed which is less sharp than the first, but is persistent.

According to Page and McCubbin (1953), this response can be divided into several parts appearing in succession:

(1) a Bezold-Jarisch effect (suppressed by atropine or transient autonomic ganglion blockade) responsible for initial hypotension;

(2) a vasoconstrictor effect exerted directly at the periphery, and causing hypertension;

(3) temporary inhibition of this effect, causing delayed hypotension.

The pressor response therefore seems "amphibaric" (a term coined by Page and McCubbin in 1953); it depends essentially on the preliminary state of vasomotor tonus. When the vessels are relaxed, the response is constrictive. Conversely, in case of high neurogenic vasomotor tonus, the inhibitory effect prevails, and vascular relaxation and hypotension result. This duality of action was demonstrated by Page and McCubbin in an elegant experiment: the perfused leg of a dog was arranged so that the sciatic and femoral nerves were intact. When the vasomotor tonus of the leg is increased by resection of the depressor nerves at the level of the aorta and carotid sinus, injection of serotonin into the perfused artery induces vasodilatation. When the nerve paths are mechanically or chemically severed (with hexamethonium), vasoconstriction occurs, caused by the direct action of 5-HT on the muscle sheath of the vessel.

Intravenous injection of serotonin (into the external juglar vein, in doses of 0.03 to 1 mg) simultaneously causes an increase in carotid blood pressure, and peripheral vasoconstriction, demonstrated with the perfused leg technique (Binet and Burstein, 1955 [23a]): it is expressed by higher pressure at the origin of the vessels. The blood of the animal acquires vasoconstrictive properties. Intra-arterial injection (of 20-50 μg into the artery of the leg) causes vasodilatation of variable intensity, even when the leg has been enervated; the latter action was accounted for by local release of histamine induced by 5-HT.

Burstein confirmed in 1956 [49b] that serotonin directly injected into the perfused area (left leg, small intestine, or myocardium of the dog) caused hypotension: it does not therefore have a direct vasoconstrictor effect. Injected in other ways, it brings an increase of blood pressure in the perfused area, thus revealing the origin of the vasoconstrictor effect in the nervous centers. This argument refutes the hypothesis concerning the role of serotonin in hemostasis. According to Emanuel et al. (1958 [81]), direct injection of 5-HT (10 μg/minute) into the brachial artery of the dog contracts the artery of the anterior leg, dilates the small vessels, and sometimes contracts the veins.

Many experiments have attempted to determine the mechanisms with precision: we have seen that vagotomy doubled, while atropine or transient ganglion blockade eliminated the initial depressor effect.

Destruction of the vasomotor center by pithing through the cervical cord stabilized the blood pressure response to serotonin in the dog (Freyburger et al., 1952 [95]).

In the opinion of Smith and Smith (1955 [239]),

5-HT-induced hypotension is not corrected by vagotomy or by antihistamines. It is potentiated by cocaine, attenuated by dibenamid, yohimbin, ethyl-2-methyl-3-nitro-5-indole. The authors suggest the theory of heparine antagonism.

After exclusion of the carotid sinus, tetraethylammonium chloride blocks the depressive response to intravenous 5-HT. 5-HT thus appears to be a very active stimulant of chemoreceptors, more potent than lobeline (McCubbin et al., 1956 [158]).

In the opinion of Heymans et al., 5-HT acts directly on the pressoreceptors of the carotid sinus.

Woolley and Shaw (1957 [277c]) believe that the effect of preliminary administration of antimetabolites, such as BAS-phenol, to animals proves the possible existence of different receptors for 5-HT on the one hand, and for tryptamine and the catecholamines on the other.

Continuous intravenous injection of 5-HT in doses of 150 μg/kg per minute causes a 28% drop in the general blood pressure of the dog, although cardiac output is increased by 60% (Rudolph and Paul, 1957 [218a]).

Blood pressure response to 5-HT in an unanesthetized dog is potentiated by methylphenidate and antagonized by reserpine (Rutledge et al., 1959 [327]).

The dog without medulla and adrenals presents a very unstable blood pressure, maintained only by peripheral vasomotor tonus; it constitutes therefore a very sensitive reagent for the study of the vascular action of 5-HT. Injected intravenously in doses of 0.03 to 0.10 mg/kg, serotonin at 5% induces hypotension with bradycardia. But in a dog that was also vagotomized, marked hypertension is observed, expressing the peripheral vascular action of 5-HT (Thieblot and collab., 1957 [248]).

In the unanesthetized dog, response to 5-HT is greatly disturbed by the cardio-inhibitor activity of the vagus (McCubbin and Page, 1957).

(*b*) In the cat and even more in the rabbit, the most usual response to 5-HT is hypotension; the Bezold-Jarisch effect and inhibition of neurogenic vasoconstriction seem to be predominant (Page and McCubbin, 1953).

A hypotensive effect in the rabbit was recently confirmed by Lecomte and Van de Berg (1958 [148a]): intravenous 5-HT, in doses of 25 μg/kg and up to 250 μg/kg, lowers carotid blood pressure, with parallel vasoconstriction of cutaneous and pulmonary arterioles. Above 300 μg/kg, only the duration of the phenomenon is increased. Sometimes hypertension is preceded by a very brief (20 to 30 seconds) and moderate (3 to 4 cm of Hg) increase in pressure. The depressor effect is only partially abolished by atropine or vagotomy. Complex pressor-depressor reactions after intravenous injection of 5-HT occur in the bivagotomized unanesthetized cat with denervated carotid sinuses. These effects are modified into straight pressor action under the influence of ganglion blockage with pendiomide or decamethonium, or of transsection of the spinal medulla (Trzebsky and Jung, 1958 [336]).

(*c*) In the rat, Correale (1954 [61]) noted that subcutaneous injection of 5-HT (0.4 mg/kg, or more) induced hypotension. Intravenously injected 5-HT, in doses of 2 to 5 μg, causes an initial mild and temporary hypotension, followed by slight hypertension, and by secondary and more marked hypotension (Salmoiraghi and Page, 1957 [219b]). Vagotomy abolishes the Bezold-Jarisch effect (initial hypertension), but not the prolonged depressive response. As in the cat, only considerable pressor response persists after ganglion blockage.

The cardio-vascular action of 5-HT thus shows the rat closer to the cat than to the dog.

(*d*) In man the vascular effects of 5-HT are similar to those observed in the dog (Page). Intravenous injection of serotonin usually causes a transient increase in arterial pressure, 5 to 7 cm Hg, associated with equally temporary hyperpnea, intestinal pains, and generalized pruritus. The blood pressure comes back to normal within 10 minutes (Snow et al., 1955). Serotonin raises local venous pressure starting with 0.3 μg/minute (Magalini and Stefanini, 1956 [161a]). The same effect can be observed after intravenous injection of lysed human platelets, but not of intact platelets. Intra-arterial injection of 5-HT raises pressure in the homolateral veins (id.). Intravenous 5-HT has a variable effect on arterial pressure, but always causes tachycardia (Hollander et al., 1957 [127]). This effect can be blocked with BAS, if administered intravenously.

According to Spies and Stone (1952, intravenous injection of 0.5 to 5 mg of 5-HT consistently causes a rise in systolic and diastolic pressure, whatever the preliminary level of arterial pressure was; in short, it occurs in hypotensive, hypertensive, and normal subjects alike. Duration of the effect is 5 to 10 minutes. It is reproducible after an interval of 2 to 3 hours. We concur with Erspamer (1956 [84] p. 136) who described the blood pressure reaction to 5-HT as highly unpredictable and irregular, and considered this substance neither as a pure hypertensive nor as a pure hypotensive agent. All depends on the choice of animal, and on experimental conditions.

(*e*) In the cock, 5-HT induces a drop in carotid blood pressure, without concomitant cutaneous vasodilatation or increase of capillary permeability, which distinguishes it from the histamine effect. This hypotension is suppressed with BOL-148 (Lecomte and Beaumariage, 1957 [147]).

Effects on the Heart

(*a*) Mammals. The effects of 5-HT on the heart are not very distinctive in mammals, and are generally expressed by stimulation (Page). This action has been studied in the isolated heart (or fragmented organs), and in the heart in situ.

1. Isolated heart. Dog. The perfused isolated heart of the dog reacts to large doses of 5-HT with augmentation of frequency, and a mild increase of amplitude (Schneider and Yonkman, quoted by J. Levy [151]). On the other hand, 5-HT does not increase the contractile power of the papillary muscle isolated from the right ventricle (Loubatières et al., 1958 [157]).

Cat. A positive chronotropic and inotropic response was obtained in the isolated cat heart by Reid (1952), and by Schneider and Yonkman (1954). Contractile power of the isolated papillary muscle was not increased (Loubatières et al., loc. cit.).

Rabbit. Freyburger et al. observed an intense positive inotropic and chronotropic response in the isolated rabbit heart (1952 [95]). Progressive abatement of this response (tachyphylaxis), reported by these authors, has been confirmed many times. Bulle (1957 [48]) noted an intense, but transient chronotropic and inotropic response in the rabbit heart, with a 10% increase of coronary output, when 5-HT was introduced via aortic cannula. When 5-HT was injected into the wall of the left ventricle (0.05 ml), he observed myocardial lesions, which can be prevented with chlorpromazine. In the isolated rabbit atrium, J. Levy and Michel-Ber (1956 [152]) obtained the following diphasic action from 5-HT: negative inotropic and chronotropic depressive action, to which atropine was antagonistic and positive inotropic and chronotropic stimulant action, which disappeared when counteracted with ephedrine. Both effects were abolished by depolarizing substances in high concentrations (nicotine, phenoxycholine).

According to Loubatières (1958, loc. cit.), 5-HT does not modify frequency or contractile power in the isolated rabbit atrium, functioning automatically, nor does it act on the isolated papillary muscle of the right ventricle. 5-HT augments the rhythm, but not the contractile force of the perfused isolated rabbit heart (Loubatières et al., loc. cit.).

Guinea pig. In an isolated guinea pig atrium subected to serotonin action, J. Levy and Michel-Ber (1956) sometimes observed positive inotropic and chronotropic effects, and at other times a biphasic response, recalling the isolated rabbit atrium response. Tachyphylaxis is also manifest.

2. Heart in situ. We already observed that under the influence of serotonin, bradycardia associated with initial hypotension occurred in the anesthetized dog, and hypotension alone in the cat. The amine, injected into the veins of the dog in doses of 150 μg/kg per minute, increases cardiac output by 60% (Rudolph and Paul, 1957 [218a]). Maxwell et al. (1957 [167]) obtained coronary and peripheral vasodilatation, but oxygen consumption by the myocardium was unchanged. According to Loubatières et al. (1958, loc. cit.), 5-HT increases contractile power, and temporarily curbs the rhythm of the dog heart in situ.

Concerning cardiac output, Olmstead and Page (1957, London) noted in the anesthetized dog a biphasic action (transitory reduction, followed by distinct, moderately prolonged augmentation), paralleling pressure variations.

(*b*) Mollusks. Serotonin action on the mollusk heart deserves to be considered. It affects both rhythm and amplitude with such intensity that Welsh (1953 [273]) did not hesitate to raise 5-HT to the rank of a cardiac excitor neurohumor. This action varies slightly with species, at least in respect to the antagonists (Woolley and Shaw, 1956).

Because of its great sensitivity, the heart of *Venus mercenaria* is the choice reagent for biologic determination of serotonin (see Chapter I).

Effects on Local Circulation

Serotonin is known generally to exert a constrictor effect on preparations of isolated arteries (carotid sections, and segments of arteries of smaller dimensions). This action is also manifest in certain perfused vascular areas, detached or not from the living animal (rabbit ear, leg, or hind part of dog), and makes biologic determination possible, as in the above experiments. The attention of physiologists has been principally directed toward coronary, pulmonary, and renal areas.

1. Coronary circulation. Vasodilatation of the coronary artery is obtained by injecting 5-HT into the descending branch (dog heart in situ: Schoffield and Walker, 1953; isolated cat heart: Schneider and Yonkman, 1954, quoted by J. Levy [151]), or in the aorta (Bulle, 1957).

2. Pulmonary circulation. The effect of serotonin on pulmonary vessels has been the subject of many recent studies. Reid and Rand (1951 [214]) had already demonstrated that 5-HT injected into a pulmonary artery elicits an increase of intra-arterial pressure; this does not occur with injection into a pulmonary vein. Comroe (1952, quoted by Page, 1957 [186]) observed a direct constrictive action of serotonin on bronchioles and pulmonary vessels in the cat; this action can be prevented by dihydroergotamine or by LSD 25 (Gaddum et al., 1953 [cf. 98]). 5-HT proves a more potent constrictor of pulmonary vessels in the dog and cat than epinephrin or norepinephrin (Ginzel and Kottegoda, 1953 [cf. 107]).

In man, increase of venous pressure and of pressure in the right atrium and pulmonary artery precedes decrease of pressure in the femoral arteries (Baldrighi and Ferrari, 1955) (cit. Page, 1957 [186]). Hypertension in the lesser circulation area is thus a constant effect of 5-HT.

In the dog subjected to continuous venous infusion of 5-HT, blood pressure in the pulmonary artery is doubled starting from 20 μg/kg per minute, and may be tripled for doses of 150 μg/kg per minute. Pulmonary resistance reaches 500% of control value, while peripheral resistance is lessened by 55% (Rudolph and Paul, 1957 [218a]).

Serotonin is therefore a potent vasoconstrictor of pulmonary vessels, which are considered to have little sensitivity to the majority of factors affecting vessels of the systemic circulation. In the opinion of Rose (1957 [215]), the effect of 5-HT is linked to direct pulmonary vasoconstriction. In animals in which the left ventricle is excluded and replaced by a diaphragm pump, it becomes possible to inject serotonin into the pulmonary artery, limiting its effect to the lesser circulation. Under these conditions, serotonin in doses of 2-40 μg/kg causes immediate pulmonary hypertension, and reduces blood flow. This remarkably selective effect is reproducible. It can be completely abolished by an antihistamine, phenothiazine, in doses of 0.6 to 1 mg/kg, and especially by the anti-serotonins, LSD 25 and its di-bromine derivative; it is less clearly abolished by BAS.

Borst et al. (1957 [35]) studied the action of various pharmacologic aspects on the anesthetized and tracheotomized dog (the thorax was opened for the purpose of experimentation); pulmonary vasoconstriction was obtained with epinephrin and norepinephrin, and much more clearly with 5-HT. The pulmonary vasoconstrictor effect was confirmed by Maxwell (1957 [167]), Rudolph and Paul (1957 [218a]).

According to Rudolph et al. (1958 [218b], 1959 [218c]), acetylcholine, which does not directly affect the tonus of the pulmonary vessels, constantly and clearly lessens the resistance of pulmonary vessels after continued action of serotonin in perfusion. Histamine does the same. A.T.P. exerts a less marked effect; therefore they do not act as anti-serotonins.

Carlier et al. (1958 [52]) compared the action of 5-HT (15-25 μg/kg) to that of angiotensine (0.2 to 0.5 mg/kg) in the pulmonary circulation of the dog (under chloralose anesthesia). While both agents caused considerable hypertension and vasoconstriction of the greater circulation, only 5-HT induced pulmonary hypertension and vasoconstriction.

5-HT, injected into the veins of rabbits, induced immediate rise of pulmonary arterial pressure, in proportion to the injected dose. At 250 μg/kg and above, this pulmonary hypertension is prolonged for 8 to 10 minutes (Lecomte and Van de Berg, 1958 [148a]).

Several authors, among whom Comroe et al. (1953) and Page (1948), had suggested a relation between the symptoms of pulmonary embolism and release of serotonin by the clot. This question was taken up again by Smith and Smith (1955 [239]).

According to Niden and Barclay (1959 [320]), serotonin increases oxygen saturation of arterial blood in the dog.

3. Renal Circulation. We have known since Page (1952) that serotonin perfused into the renal artery of the dog caused an important vasoconstriction. The phenomenon is less evident in the cat (Reid, 1952; Ginzel and Kottegoda, 1953; quoted by J. Levy [151]). The known anti-diuretic properties of 5-HT (Erspamer, 1954 [83]) contributed to center attention on the renal vessels. However, we shall examine the effect of 5-HT on diuresis later, and limit ourselves here to the study of renal blood output. Abrahams and Pickford (1957 [192]) concluded from their experiments on conscious dogs that 5-HT injected into the systemic circulation had no particular action on renal vessels. According to Page and Glendening (1955 [184a]), intravenous serotonin causes visible blanching of the kidney in rat, mouse, rabbit, and guinea pig, with a minimal dose of 0.07 mg/kg. This ischemia is mainly located in the cortex. Zones of necrosis were found in the cortex of rats sacrificed one week after injection of 4 mg/kg of 5-HT continued for 6 hours.

Page and Glendening compare experimental and clinical observations: they attribute renal lesions observed in pregnant women who died more than 24 hours after extensive abruptio placentae, to massive release of 5-HT by lysed platelets in large quantities.

Let us recall that ischemic necrosis of the renal tubes is a consequence of chronic 5-HT intoxication in the rat (Hedinger and Langemann, 1955 [121a]). While Sala and Castegnaro noted a decrease of renal plasma output, Spinazzola and Sherrod (1957 [242]) found it increased after intravenous injection of 10-20 μg/kg of 5-HT per minute in the dog. These doses did not influence the mean blood pressure. Cuypers et al. (1957 [67]) perfused the kidneys of a dog with fresh heparinized blood, and found that 5-HT, added to the perfusion liquid in amounts up to 16 μg/ml, had no measurable effect on renal output. In comparison, l-adrenalin and l-arterenol, in concentration of 0.02 μg/ml, have a potent vasoconstrictor effect. Histamine in small doses (0.2 ·μg/ml increases renal output. Emanuel et al. (1958 [81]) report that 5-HT, injected into an anesthetized dog in doses of 10 μg per minute for 5 minutes, increases the geometric component of renal vascular resistance.

It is difficult to reconcile the various experimental results obtained in the dog: nevertheless, we want to stress the fact that vasoconstriction and renal necrosis

are only obtained with relatively large doses of 5-HT, exceeding physiologic doses by far.

4. Hepatic circulation. 5-HT produces constriction of the arterial system of the liver and of the portal vein, but does not seem to be responsible for the congestion present in isolated and perfused dog liver; the congestion is particularly pronounced after perfusion with defibrinated blood (Andrews et al., 1958 [3]).

5. Circulation in limbs. Certain preparations, such as the hind part of the guinea pig or the leg of dog or cat, are useful in pharmacologic studies to compare the action of 5-HT to that of other drugs with vascular action, particularly, concerning antagonists (J. Levy, p. 906). Binet and Burstein's experiment (1955 [23a]) emphasizes the importance of the access route. Intravenous serotonin causes a simultaneous carotid hypertension and peripheral vasoconstriction; this has been demonstrated in the dog with the perfused leg technique. This vasoconstriction is probably linked to the tonus increase of the vasoconstrictor nerves originating from the centers (Burstein, 1956 [49b]).

Injected directly into the artery of the leg, 5-HT generally causes vasodilatation of variable degrees at the level of the leg, whether neural connections are intact or not. The vasodilatation, which does not seem linked to a variation in tonus of the vasomotor nerves, may be due to release of histamine in the perfused limb (Feldberg and Smith, 1953, quoted by [49b]). This theory was partially confirmed by Binet and Quivy (1958 [23b]) with experiments on the rat leg; local injection of 5-HT resulted in histamine depletion of the cutaneous tissues, with an average loss of 42.4%, compared to controls.

6. Isolated ear of rabbit and guinea pig. Intensive vasoconstriction, induced by serotonin perfused in the isolated ear of the rabbit (Green and Page, M. Zucker [284c]) or guinea pig (Grette, 1957, quoted by J. Levy [151]), allows biologic determination of serotonin, and study of its antagonists.

C. ACTION OF SEROTONIN ON THE RESPIRATORY SYSTEM

This action is complex and has at least two components (if the substance is injected intravenously): a vascular component (vasoconstriction of the pulmonary artery), and a bronchial component (action of the 5-HT on smooth muscles).

(*a*) Intravenous injection of 5-HT causes arrest of respiration followed by hyperpnea in the dog and cat, immediate hyperpnea in the rat, and sometimes asthmatiform dyspnea in the rabbit. The mechanism of this respiratory action is disputed. It can be attributed either to stimulation of the chemoreceptors or to a direct action on the centers (cf. 19, [151]). According to Rudolph and Paul (1958 [218a]) acetylcholine, while reducing pulmonary vessel resistance increased by 5-HT, does not counteract the bronchoconstrictor effect; therefore it is not an anti-serotonin.

Hollander et al. (1957 [127]) claim that 5-HT always causes hyperventilation in man, and sometimes dyspnea. This effect is blocked by intravenous BAS, but not by oral BAS. Michelson et al. (1958 [171]) gave rapid intravenous injections of 0.5 to 1.5 mg of 5-HT to 22 normal and 8 asthmatic subjects who breathed through a mouth piece into a spirometric system hooked up with two kymographs. In 27 subjects out of 30, respiration was accelerated and its amplitude increased. Transient apnea was observed, followed by hyperpnea in 3 normal subjects. The respiratory response is potentiated by intravenous pyrilamine maleate; it is not modified by anticholinergic or antiadrenergic drugs, and is inhibited by BAS.

(*b*) The guinea pig exposed to an aerosol containing 1% of 5-HT develops a shock syndrome within 60 to 150 seconds, with sneezing, followed by dyspnea, convulsions, and finally collapse (Herxheimer, 1955 [123]). This syndrome, which resembles that produced by aerosols of histamine or acetylcholine, differs from it by its usual mildness, by the development of tolerance (tachyphylaxis), and the response to specific antagonists.

D. ACTION ON THE GASTROINTESTINAL TRACT

We must distinguish between effects on the mucosa and motor effects.

1. Action on the gastrointestinal mucosa. Large doses of serotonin cause ulcers of the stomach and intestines in the rat, as histamine does (Hedinger and Veraguth, 1955, quoted by Holtz). Owens and Masson (1958 [181]) tried to determine the mechanism of acute hemorrhagic ulcers induced in the rat by endogenous serotonin release, associated or not with histamine liberation. The ulcers are limited to the glandular portion of the stomach. They are not due to an increase in volume and acidity of the gastric secretion, since these values are reduced after injection of 5-HT in oily suspension (6 mg of free base). They seem to result from a direct action of the amine on the gastric vessels. Treatment with B.A.L. (4 mg/kg) prevents the ulcers produced by a massive injection of 5-hydroxytryptophan (300 mg/kg). Exogenous serotonin, in doses of 0.01 mg/kg per minute, has no significant effect on the acid secretion of the dog's stomach, but beginning with 0.04 mg/kg, the secretion in Heidenhain's pocket becomes less acid (Haverback et al., 1957 [120b]). According to Black et al. (1958 [24]) 5-HT perfused in doses of 10 μg/kg per minute

into anesthetized dogs with gastric fistulae does not induce true gastric secretion, but only an increase of mucus volume. On the other hand, in doses of 15 μg/kg/minute, 5-HT inhibits the gastric secretion induced by histamine (5 μg/kg/minute). This inhibiting power appears 30 minutes after the beginning of perfusion and disappears about an hour after its end. It does not occur in dogs with cervical vagotomy.

2. Motor action. This is included in the general action of serotonin on smooth muscle fibers, but involves special receptors. Serotonin begins to exert a potent stimulant action on the intestinal motility of the dog at a dose of 0.01 mg/kg/minute (Haverback et al., loc. cit.). In the anesthetized rabbit, 5-HT in vivo exerts a direct and indirect cholinergic action on the small intestine (Georges and Herold, 1957 [103], 1958 [104]): the threshold dose is 25 μg/kg. The maximal effect is obtained with a dose of 0.5 mg/kg; contracture of the intestinal loop lasts 15 to 45 seconds. In the vagotomized and decerebrated rabbit, serotonin induces immediate spasm of the intestine, but without peristalsis. The intestine is particularly sensitive to the phenomenon of tachyphylaxis, or tolerance to repeated doses (Gaddum et al.).

On the isolated intestine (rat duodenum (guinea pig ileum) the effect of 5-HT seems complex to J. Levy and Michel-Ber (1956 [152]). Serotonin acts on motor receptors, and also at the level of preganglionic fibers. When added to the perfusion fluid of guinea pig or rabbit intestine, 5-HT in concentration of 10^{-5} to 10^{-9} M stimulates peristalsis, lowers the threshold of the intraluminal pressure needed to induce peristalsis, and increases contraction rate and fluid volume. This effect is reversed under the influence of LSD and BOL. When applied to the serosal surface of the intestinal lumen, 5-HT in low concentration inhibits peristalsis; in high concentration it abolishes it completely (Bülbring and Lin, 1957 [295a]). As indicated by Benditt and Rowley (1956, quoted by [27b]), the site of 5-HT activities can be located through blockage with chlorpromazine. Severe diarrhea, one of the clinical carcinoid symptoms, has been attributed to serotonin. However, its role in the regulation of intestinal motility, suggested by Udenfriend et al. [259], and by Holtz (1958 [309]) has not been demonstrated.

E. ACTION OF SEROTONIN ON THE RENAL FUNCTION

The effect of serotonin on renal hemodynamics was mentioned in an earlier chapter. According to Erspamer, this controversial effect consists essentially in intense vasoconstriction with cortical predominance.

To Erspamer and his collaborators we owe the main specific investigations on renal function (1954 [83, 84]). Four to 10 μg/kg of 5-HT in subcutaneous injection are enough to cause in the rat a marked reduction of diuresis. 5-HT slows down glomerular filtration, with regression of chloride elimination and sometimes of renal plasma output.

Since the general blood pressure is not affected, Erspamer attributes this reduction of urinary excretion either to constriction of afferent vessels and vasodilatation of efferent vessels of the kidney, or to one of these factors alone. Whatever the mechanism of this action, it imparts to serotonin the role of a hormone regulating diuresis.

Sala (quoted by Page, 1958 [187b]) goes so far as to identify serotonin with the stable antidiuretic substance in rat serum studied by Dicker and Ginsburg and by Heller. He bases this assimilation on (1) inactivation of both by diazonium salts and amine oxidase, (2) reduction of glomerular filtration rate by constriction of the afferent arterioles, (3) simultaneous appearance in serum during coagulation, and (4) correlation between ADS activity and the 5-HT content of serum. This concept is opposed by Abrahams and Pickford, who in the first place observe a great difference between dog and rat responses to subcutaneous injections of 5-HT: it takes 74 μg/kg (in serotonin base) to obtain the same antidiuretic effect in the dog as from 4 μg/kg in the rat. As the antidiuretic effect is not significantly influenced by venous vs. arterial introduction into the general circulation, Pickford excludes a central origin. On the other hand, reduction of diuresis is observed even after denervation of the kidney (Pickford, 1957 [192]).

Spinazzola and Sherrod, already quoted [242], observe a distinct diuretic effect of 5-HT in continuous intravenous infusion into the unanesthetized female dog, beginning at 10 μg/kg. The effect is not increased above doses of 20 μg/kg. Glomerular filtration is reduced from 15 μg/kg on.

Oliguria, observed in the dog, is not due to renal ischemia (Nizet, Wilsens and Barac, 1953). Barac (1955 [10]) further studied the effect of 5-HT on the renal function of the dog as part of his investigation of burns; he observed that 5-HT decreased diuresis and chloruria in the intact dog. It decreases diuresis, but does not raise chloruria in the hypophysectomized dog. He concludes that 5-HT has a direct action on the kidney, without intervention of the pituitary body. According to the same author (1957 [11]), LSD and BOL—a dibrominated derivative—are capable of autonomous antidiuretic action comparable to that of 5-HT; they do not prevent the antidiuretic effect of burns.

Observing the effect of small doses of intravenous serotonin in a cat under light chloralose anesthesia, Fastier and Waal (1957 [302]) did not think that the

depressive reflex intervened in the reduction of urinary output.

The effect of 5-HT on renal function has also been studied in man. We shall quote Notter (1956 [180]): serotonin, administered in subcutaneous or intravenous injection (5 mg in 5 minutes) does not modify paraamino-hippuric acid clearance. Cora et al. (quoted by Page, 1958) state that 5 mg of intravenous 5-HT have no effect on diuresis or glomerular filtration rate. Hollander et al. (1957 [127]) found that injection of 1 mg serotonin into 18 hypertensive subjects temporarily reduced renal plasma output and diuresis by 30%, without necessarily altering glomerular filtration rate. 5-HT therefore acts as a mild diuretic. Page himself and his collaborators observed that serotonin injected into normal subjects reduced renal output and glomerular filtration rate, while mean pressure was slightly increased. It has also been suggested (Hulet and Perrera, 1956; Page 1958) that serotonin intervened in the metabolism of sodium (increased retention) and of potassium (increased excretion). Dibenamine, a serotonin antagonist, inhibits the antidiuretic action of 5-HT, if it is administered before serotonin (Erspamer and Correale, 1953). When injected at the same time or later, it increases the antidiuretic effect. The authors' interpretation of this fact is criticized by Van Arman (1956); he confirms the facts, but does not exclude an independent antidiuretic action of dibenamine.

The action of 5-HT on the perfused isolated dog ureter is different from its action on the intestine. (Borgstedt, 1959 [292]). 5-HT added to the bath or to the perfusion fluid in concentrations of 1.10^{-11} M to 2.10^{-4} M does not induce contraction of the ureter. But in the presence of LSD, a dose of 0.7. 10^{-7} M or more induces a response of the ureter to the elevation of pressure. The response to LSD is not inhibited by 5-HT, nor by diphenhydrazine, atropine, or dihydroergotamine in average concentrations. Serotonin does not inhibit spontaneous contractions.

Interaction of Serotonin and Catecholamines

Similarity of chemical structure and sensitivity to monoamine oxidase invite comparison between serotonin and the adrenalin-noradrenalin group.

We know that 5-HT in large doses enhances the hypertensive effect of adrenalin (Freyburger et al., 1952 [95]), and that it acts as an antagonist to the toxicity, piloerection, and bradycardia induced by noradrenalin (Gordon et al., 1958 [110a]). In the mouse (id., 1959 [110b]) serotonin inhibits the piloerection induced by adrenalin and noradrenalin. It reduces adrenalin-induced pulmonary edema in the rat, rabbit and mouse. On large segments of artery, serotonin and noradrenalin act as synergists; on arterioles they act as antagonists. Intramuscular 5-HT inhibits the delayed rise of hepatic glycogen induced by intraperitoneal adrenalin in the fasted rabbit. Gordon et al. (1958 [110a]) also studied modifications subsequent to adrenalin perfusion of the dog forepart, with and without serotonin. 5-HT always increased the output of the perfused limb; adrenalin reduces the output. Blood pressure increase induced by adrenalin is always greatly enhanced by serotonin. These effects are less constant and distinct with noradrenalin.

Injection of adrenalin elicits a bradycardiac reflex. 5-HT changes it into tachycardia. 5-HT thus limits the peripheral action of noradrenalin on blood vessels and the action of both catecholamines on the carotid sinus. Schmitt and Schmitt (1959 [330]) confirm with added detail the observations of Lecomte (1955) and of Costa and Zeller (1958): in small doses, 5-HT attenuates the effects of adrenalin and noradrenalin on the nictitating membrane of the cat; in large doses, it potentiates these effects.

According to Buzard and Nytch (1958 [296]) noradrenalin acts as a non-specific, non-competitive inhibitor of 5-HTP decarboxylase in the kidney of the cat.

On the whole, we find more opposition than convergence in the actions of serotonin and the two adrenalins. Benda (1959 [17d]) mentioned the concept that serotonin was a hormone of the trophotropic system, in contrast to noradrenalin, a hormone of the ergotropic system of Hess.

CHAPTER V

Toxicity—Side Effects

Toxicity

Acute toxicity is not great; in the mouse, LD/50 is obtained with intravenous injection of 160 mg/kg, or subcutaneous injection of 868 mg/kg;[7] in the rat, with 30 or 117 mg/kg, depending on the injection route (Freyburger, et al., 1952 [95]). According to Lecomte et al. (1958), the intravenous lethal dose in the rabbit is larger than 5 mg/kg.

Few studies have been made on chronic toxicity. However, rats are known to be unaffected by subcutaneous doses of 1 mg/kg of serotonin, administered for 1 to 4 months without any repercussions on general

[7] Mice show slight sensitivity to serotonin; they can be given in peritoneal injections the massive doses (100 mg/kg) necessary to achieve the passage of 5-HT into the CNS (Shore et al., 1957 [234]).

condition, blood pressure, or internal organs. Large subcutaneous doses—such as 5 to 15 mg/kg for 10 to 40 days—are needed to cause severe disorders, mainly ischemic necrosis of the renal tubes (Erspamer, 1956 [84]). According to Hedinger, renal lesions are eventually caused in rats by the massive doses needed to increase the number of platelets. When rats are given subcutaneous injections of 50 mg/kg of 5-HT every other day for a period of 60 days, intoxication is evidenced by prostration, convulsions, and diarrhea from the first days of medication; in the surviving animals (3 out of 12 die) the signs of intoxication become attenuated, but weight fails to increase.

In young rats which had received daily doses of 16 mg serotonin subcutaneously for almost a year (353 days), MacDonald et al. (1958) observed certain morphologic effects stemming from chronic toxicity. The maximal dose given to an animal was 5,434 mg. Following this prolonged administration, many organs showed morphologic alterations such as skin fibrosis, necrosis of the tip of the tail, necrosis of renal tubes and cortex, and opacity of the cornea. All these lesions can be attributed to a potent local vasoconstrictor effect. Renal lesions occurred earlier in rats subjected to a cirrhogenous regimen (see also: MacDonald, 1959 [159b]). But the authors found neither lesions of the cardiac valves—a classic element of the carcinoid syndrome—nor the gastrointestinal ulcers likely to result from an excess of endogenous serotonin (cf. Owens and Masson, 1958).

SIDE EFFECTS OF SEROTONIN

A. Pain-Producing Effects of Serotonin

Armstrong et al. (1952 [5]) called attention to the algogenic property of serum and 5-HT, when applied to the skin at the base of a cantharidal blister. Pain occurs after a latent period of 10 to 45 seconds, and usually lasts for a few minutes. The exposed skin area becomes refractory to further applications of 5-HT or serum; but remains sensitive to acetylcholine. 5-HT in the form of creatinine sulfate induces pain in concentrations up to 10^{-6} g/ml, while tryptophan does not cause pain in concentrations of 10^{-5} to 10^{-3} g/ml. In the course of further investigations, Armstrong [7] found that the algogenic substance which forms in an exudate fluid is also present in platelet-free plasma 10 minutes after contact with the glass, no matter what anticoagulants are added. Like 5-HT it is characterized by delayed and lasting pain.

The algogenic property of 5-HT must be remembered in clinical practice. When administering transfusion of lyophilized platelets according to the technique of Klein et al. (1956), the authors frequently observed a sharp pain reaction along the venous route. It can be legitimately attributed to serotonin released by the platelets; pain does not occur, or only in mild degree, when the platelets have been washed in an isotonic saline solution before the transfusion, as suggested by M. Zucker (personal communication). We observed a very distinct but fleeting pain reaction in a few transfusions of fresh platelets in high concentrations; the pain yielded to hot compresses. We do not know whether a venous constriction occurred due to serotonin, or a local reaction usual in patients subjected to multiple transfusions.

B. Fever-Inducing Effect

Horita and Gogerty (1958 [128]) observed a pyrogenic action of 5-hydroxytryptophan administered to the rabbit. This action is largely attenuated by preliminary administration of the bromine analogue of LSD (BOL-148).

C. Protective Effects

Laborit and Broussolle (1957 [143a], 1958 [46]) observed in the mouse a protective action of 5-HT against convulsions induced by pressurized oxygen. They attributed this fact to the antioxidizing properties shown by serotonin at CNS-cell level. Along the same line, Van den Brenk and Elliott (quoted by Laborit et al., 1959 [143d]) found that serotonin had a protective role against ionizing radiations. Rauchenbach and Tchernov (1959 [325]) observed variations of serotonin levels in irradiated animals; 5-HT gradually decreased in the blood until it disappeared; this was not linked as might be expected to rarefaction of the platelets, for the serotonin level climbed again during the terminal period.

D. Effects on Growth

It may be artificial to compare the phenomena of vegetal growth and animal growth. However, Niaussat et al. (1958 [179a, b]) demonstrated the effect of serotonin on the growth of young oat sprouts. The activity of serotonin in various concentrations was tested on cell elongation in oat sprouts, according to Went's classic "avena test." For serotonin concentrations of 10^{-5}, 10^{-6}, 10^{-7}, the mean curvature angles of the coleoptiles reveal a significant cell elongation; indoleacetic acid was used as control.

Massive increase in urinary excretion of 5-HIA acid after injection of somatotropic pituitary hormone into the rabbit suggested to Laborit et al. (1959 [143c, d]) an important biologic role of serotonin. This urinary elimination is depressed by iproniazid.

In the rat, serotonin causes a decrease of thyroid activity, as shown by Zizine (1959 [339]) in ex-

periments achieved with the aid of I^{131}. Sirek made a comparable observation (1957 [333]): 5-HT exerts on a depancreatized dog a hyperglycemic effect not observed in the normal dog, and similar to the effect of growth hormone in diabetic subjects. In physiologic or even massive doses (10 mg/kg), 5-HT does not increase glycemia in the rabbit and rat.

E. Effects on Blood (except platelets)

5-HT exerted no effect on peripheral blood picture or on glycemia in 21 human volunteer subjects (Scaltrini, 1956 [222]). Steiner and Hedinger (1945 [245]), however, observed eosinopenia parallel with thrombocytopenia, becoming very serious 12 hours after injection of a dose of 250 micro-moles per kg.

A single subcutaneous injection of 2 to 20 mg/kg into the rat results in marked decrease of eosinophiles in the circulating blood after 8 hours. In the next few hours, a dose of 50 mg/kg has no further effect. Serra and Bruni (1957 [224]) attribute the eosinopenia to liberation of A.C.T.H. In the adrenalectomized rat, injection of a single dose of 5-HT produces eosinophilia; maximal values of this eosinophilia do not coincide with those of thrombocytosis (Steiner et al., 1957 [246]). In man, injection of 5-HT (5 mg intramuscular, or 30 gamma intravenous) induces a fleeting eosinopenia (Rosa et al. [325 bis]). In the rabbit, 5-HT significantly raises the basophile count after 24 hours (Bosella, *Experientia*, 1959, 25: 343-344). In concentration of 10^{-4}, 5-HT increases the phagocyte activity of polynuclears in rat and rabbit by approximately 25% (Ludany et al., 1958 [315]).

CHAPTER VI

Serotonin Antagonists

The study of serotonin antagonists is primarily related to pharmacology since the modus of action of this amine was determined by differentiating it from other drugs acting on smooth muscle. This study, however, was spurred on by the importance of the clinical effects attributed to serotonin—perhaps with some exaggeration—and by the hope of therapeutic application.

We do not claim to draw up a complete list of 5-HT antagonists, especially when many of them can be defined by their action on the central nervous system (Cf. Woolley and Shaw [277c], Page's review [187b], Bijlsma and Ernst's report, 1958 ([22]). Yet it is useful to know them to be able to interpret the effects of serotonin as a whole.

Along with Erspamer (1956 [84]) and with Poumailloux and Labignette (1959 [199a, b]) we shall distinguish:

(1) a group of indoxl-alkylamines including gramine, whose methyl-2-chloro-5-gramine derivative is particularly active [8]; their action is best observed in vitro, e.g., on preparations of estrual rat uterus;

(2) products active in vitro and in vivo: in the first place, lysergic acid diethylamide, or LSD 25, most potent and complete of the known serotonin antagonists, at least until recent years (cf. Gaddum, 1954; Erspamer, 1954);

(3) certain sympathicolytic agents, dibenamine and dibenzyline, equally active in vitro and in vivo;

(4) alkaloids (yohimbine and harmine alkaloids), which are rather weak antagonists; and finally,

(5) synthetic products, primarily studied by Shaw and Woolley [227, 228].

Rauwolfia serpentina alkaloids deserve a study per se, especially reserpine which is of considerable interest for the study of serotonin. A certain chemical relationship exists between reserpine and yohimbine. In fact, they seem to act as serotonin liberators rather than antagonists, since they have no in vitro action. Nitroindole analogues do not antagonize the in vitro action of serotonin on carotid sections as do the corresponding aminoindoles (Woolley and Shaw, 1957 [277c]). Reserpine, chlorpromazine and Frenquel (tranquilizers) inhibit the 5-HT induced contracture of the uterus; lysergic acid and mescaline (hallucinogens) act as synergists. 2-methyl-3-ethyl-5-nitroindole,* an antimetabolite of serotonin, acts in vivo only after 12 to 48 hours.

Shaw and Woolley (1956 [227]) proved the existence of several methyl derivatives of serotonin, which retain certain functions of serotonin but are nevertheless capable of acting as antagonists: 2,5-dimethylserotonin, a water-soluble substance active on isolated arterial sections and uterus, antagonizes the pressor action of serotonin in the dog (1 mg protects against 0.5 to 1 mg of 5-HT). Among many other derivatives assayed, 1-benzyl-2,5-dimethylserotonin, or 1-benzyl-2-methyl-5-methoxytryptamine, proved to be a potent antagonist even in oral administration to the dog. To this new compound Woolley and his collaborators gave the name of BAS (Benzyl Analog of Serotonin) which characterizes it as a perfect antimetabolite able

[8] According to Quadbeck and Röhm (quoted by Page [187b]) who prepared and assayed a series of 31 substituted gramine derivatives.

* In French text: 2-éthyl-3-méthyl-5-nitro-indole. [Translator's correction.]

to displace serotonin from its sites. In oral administration its action is not immediate; it requires a delay of 12 to 24 hours. A closely related and no less active compound is BAS-phenol, or 1-benzyl-2-methyl-5-hydroxytryptamine; its synthesis was achieved by Shaw and Woolley (1957 [228]).

BAS administration causes a slight reduction of serotonin content in the stomach and small intestine of the mouse.

The 5-HIA content of urine is unchanged while the excretion of 5-HT is markedly increased; BAS may thus be considered to inhibit monoamine oxidase (Woolley and Edelman, 1958). In this respect it behaves like iproniazid (1-isonicotinyl-2-isopropylhydrazine), which prolongs the action of serotonin injections in animals, evoking experimental hyperserotoninemia. In the carcinoid syndrome, iproniazid in daily doses of 50 to 100 mg increases flushing and diarrhea, well-known symptoms of hyperserotoninemia, while the 5-HIA content of urine is significantly decreased (Kabakow et al., 1958 [136]).

LSD in a very weak dose (0.005 μg/kg) seems to potentiate 5-HT action on the contractions of estrual rat uterus while inhibiting it in a strong dose (0.5 μg/kg). Delay and Thuillier (1956 [73a]) compare this dual action to that of nicotine on ganglionic fibers. Conversely, 5-HT potentiates the effect of LSD on the activity of the Siamese Fighting Fish (Trout, 1957 [335]). LSD blocks the pressor-depressor effect of serotonin in the rat (Salmoiraghi et al., 1957 [219a]). The bromine derivative slightly inhibits vasoconstriction in the 5-HT irrigated leg.

BOL lowers blood pressure in the anesthetized rat and less significantly in the cat and dog. Orally, it is inactive.

BOL inhibits the pyrogenic effects of 5-hydroxytryptophan injected in large doses into the rabbit (Horita and Gogerty, 1958 [128]); this effect of 5-HTP is probably due to its penetrating into the brain tissue where it produces 5-HT. A very similar effect of LSD is also inhibited by BOL.

Buchel and J. Levy (1958 [47]) studied the antiserotonin properties of eight synthetic amino ethers, containing the radical hypotensors 1-cyclohexyl-cyclohexyle, and 1-phenyl-cyclohexyle.

Antagonism of serotonin and noradrenalin in vitro has been mentioned (Gordon et al., 1958 [109, 110]); the two substances will sometimes combine their effects, and sometimes compete.

Morphine and some other analgesics inhibit 5-HT activity on the isolated guinea pig ileum (Medakovic, 1958 [168]). In the conscious rat, it reduces the intestinal peristalsis stimulated by 5-HT (id. [169]). Conversely, 5-HT directly injected into the common carotid of the dog under chloralose anesthesia causes a strong and lasting resumption of respiration previously depressed by a large dose of morphine (id. [170]).

Heparin inhibits or eliminates 5-HT-induced contractions in the isolated rat colon or uterus (Kerrer, 1957 [137]); it does not modify histamine-induced contractions. The same author claims that serotonin and histamine in large doses reduce the in vitro antithrombin action of heparin (Keller, 1958 [138]). There is, however, no proof of a direct antagonism between heparin and 5-HT in man (Lambert, 1958 [313]).

We shall name in conclusion a few therapeutic applications of serotonin antagonists: BAS administered to hypertensives reduced blood pressure in one-fourth of the subjects (Wilkins and Hollander, 1957 [276b]). BAS and LSD do not inhibit the vascular effects of serotonin massively released in carcinoid tumor patients (Schneckloth et al., 1957 [223]). Chlorpromazine and intravenous bromolysergide can partially counteract vascular and visceral injuries due to an excess of serotonin. Unfortunately, none of these products is manageable enough to be retained for therapeutic use.

In contrast to chlorpromazine (RP 4560), both a potent antihistamine and antiserotonin, prochlorperazine (RP 6140), and levomepromazine (RP 7044), are less active towards serotonin in the rat uterus test (Courvoisier et al., 1957 [63]).

CHAPTER VII

Serotonin and Blood Platelets; Role in Hemostasis

History

The discovery of the vasoconstrictor factor of serum and its identification have often been related. They may be described in the three following stages: (1) Demonstration of a vasoconstrictor activity of unknown nature; (2) Chemical identification; (3) Investigation of the physiologic role of serotonin.

1. O'Connor (1912) and F. T. Zucker (1913) had already established a relation between blood platelets and the appearance of vasoconstrictor action. Hirose (1918 [125]) stated that the vasoconstrictor effect of defibrinated human blood on isolated beef carotid was roughly proportional to the platelet count of the circulating blood. T. C. Janeway and his collaborators (1918 [134]) observed a potent vasoconstrictor effect in an extract of beef, dog, and pig platelets. The circulating plasma of the same animals had no action on the isolated carotid. Klecki and Pelczar (1926 [140]) made a systematic study of the disintegration

products of platelets and their complex physiologic action. G. Reid (1943 [210]) found that platelets played a part in hemostasis by releasing an active substance during lysis. This substance was named "thrombocytin" (the term "thrombotonin" was also used). In 1942, G. Reid and M. Bick (213) had confirmed Janeway's observation that during blood coagulation a vasoconstrictor substance passed into the serum; this substance, which acted on smooth muscle, had been preformed in the platelets.

In the following years it became more and more apparent that the substance extracted from desiccated rabbit serum and from the "buffy coat" of human blood had individual characteristics. M. Zucker (1946 [279]) followed by Reid (1947 [211]) differentiated this substance from histamine and from other known vasoconstrictors and vasodilators.

M. Zucker (1946 [279], 1947 [280]), studying the reactions of mesenteric vessels in the rat, attributed the constriction of adjacent vessels to a diffusible factor released by the platelets enclosed in the thrombus.

2. An important step was achieved by Rapport (1948 [205]) when he isolated from beef serum a vasoconstrictor substance in crystalline form. He called it serotonin and gave its chemical structure (Cf. table I).

Extensive research developed on this strong chemical foundation. Rand and Reid confirmed the platelet origin of serotonin in beef and rabbit serum (1951 [203], 1952 [204]). Improved techniques of dosage and identification enabled Bracco and Curti to assert the presence of serotonin, or 5-hydroxytryptamine, in the platelets of rabbit and sheep (1953 [36]) and of dog and man (1954 [37]), and in the thrombocytes of birds (goose and chicken) (1956 [39a]).

Moussatche (1954 [174]) differentiated serotonin from thrombocytolysine.

3. The role of platelet serotonin in hemostasis was systematically studied by M. Zucker (1951 [284a, b], 1955 [285]), Erspamer (1956 [84]), Vercellone and Asero (1954 [264]), Hardisty and Stacey (1956 [117]), Wiener and Udenfriend (1957 [275]), and recently by M. Zucker (1959 [289]). Relations between blood platelets and serotonin are so close and still pose so many problems, that they must be given a specific study. We shall examine successively:

(1) Levels of circulating serotonin in man and mammals under normal conditions;

(2) binding of serotonin in the platelets;

(3) liberation of serotonin from the platelets;

(4) experimental action of 5-HT on the number of platelets and

(5) on clotting mechanisms in vitro;

(6) serotonin in hemorrhagic diathesis;

(7) finally, role of this amine in animal and man according to experimental and therapeutic evidence.

(1) NUMERICAL DATA

It must be noted that the values quoted in the literature vary with the methods of assay. On the same acetone extracts, Polli et al. (1958 [197]), obtained markedly higher figures with spectrophotometry than with spectrofluorometry. However, the figures remain within the same range.

(*a*) In normal man.—Detailed figures were given in Chapter II (Distribution). Whole blood and serum contain an average of 0.10 μg 5-HT per ml. The total circulating blood of an adult thus contains approximately 0.5 mg serotonin.

While the circulating plasma is practically devoid of serotonin (under conditions described above), the total 5-HT content of the blood is to be found in the platelets. One billion platelets—the platelet count of about 4 ml blood—yielded 0.3 to 0.5 μg serotonin; and 1 ml blood contains about 250.10^6 platelets, i.e., one-fourth of that amount of serotonin, or 0.1 μg.

(*b*) Animals show considerable differences between species. When the 5-HT content is viewed both in whole blood (or serum), and per platelet unit, some progressive pattern seems to evolve. The guinea pig and rat are lowest (with values slightly higher than in man). The dog has 0.25 μg 5-HT per ml of blood, 1.7 μg per billion platelets. The rabbit is well supplied in serotonin: 4 μg per ml of blood, and 7.5 μg per billion platelets (Humphrey and Jaques, 1954 [130b]). Rabbit platelets are similarly rich in histamine. The serotonin content of the platelets is by far the highest in the whole organism, as indicated by Hardisty and Stacey (1955 [115]); it is about 5 times higher than in the richest area of the intestinal mucosa in the dog and rabbit (Feldberg and Toh, 1953 [89]), and twice higher than in the rabbit spleen (Erspamer, 1954 [83]).

It should be noticed here that the spleen, which is poor in decarboxylase, owes its richness in serotonin to the local destruction of a large number of platelets.

(2) BINDING OF 5-HT IN THE PLATELETS

It seems to be well established that 5-HT is not formed by the platelets themselves. However, this theory which comes to mind naturally has had its defenders. Udenfriend and Weissbach (1954 [256]) injected rats with C^{14}-labeled tryptophan and observed a half-life of 2.5 days for the labeled 5-HT, coinciding with generally accepted data for the half-life of platelets. 5-HT may therefore be incorporated in the platelets from the time of their origin. But no other experimental fact is known to support this theory. We lack in particular exact data on the 5-HT content of bone marrow, where platelets originate, and on the presence of 5-HT-tryptophan-decar-

boxylase in platelets or in megakaryocytes. On the other hand, many experiments seem to prove the platelets' ability to absorb the 5-HT supplied by the surrounding tissues. If we admit this important fact, we must define its mechanism and localization.

A. The ability of blood platelets to take up serotonin has been demonstrated in vitro and in vivo.

(*a*) in vitro—Humphrey and Toh (1954 [130a]) observed that dog platelets washed with an isotonic saline solution absorbed 5-HT at laboratory temperature, but not histamine. According to Hardisty and Stacey (1955 [115]), human platelets absorb 5-HT only at a temperature of + 37°C. Zucker and Borelli (1956 [288]) studied the absorption of serotonin (in a concentration of 0.2 to 0.4 μg/ml) by dog and human platelets. Apparently a normal morphologic condition of the platelets—a discoid shape—is required for the absorption; when the platelets take on spinous or spherical shapes in extravasated blood, absorption becomes less regular. Absorption is therefore higher in plasmatic than in saline milieu. Born and Bricknell (1958 [32b]) made a volumetric study of absorption by the platelets in serotonin-rich plasma ($\geq$ 1μg/ml).

(*b*) in vivo—McIsaac and Page (1959 [160b]) traced the fate of C^{14}-labeled 5-HT in the organism of rats and rabbits. After 2 days, 3.25% of total injected activity was taken up in the circulating platelets; after 3 days, 3.3 ± 1.7% was still found, without affecting the strong activity lost in the urine. The absorbed serotonin thus seems protected from metabolism and elimination for several days.

The affinity of the platelets for exogenous serotonin contrasts with the lack of affinity of the red blood cells (Stacey, 1956 [quoted by 160b]).

Saturation is an interesting concept. The platelets are in no way saturated in vivo, for if they are suspended in normal plasma they absorb serotonin added in vitro. When serotonin concentration in plasma is 0.2 μg/ml, the platelets can bind up to 0.38 μg per billion. When serotonin concentration in the surrounding tissue reaches 2 μg/ml, absorption does not exceed a level of 2.8 μg per billion platelets. Kuntzman and his collaborators (1956 [141]) observed that the platelets of reserpine-treated guinea pigs lost their ability to take up serotonin in vitro.

B. Mechanism of the uptake. The platelets are known to have the property to take up a whole set of substances which may occur in the circulation, such as adrenalin and noradrenalin (Weil-Malherbe and Bone, 1954-1958), histamine under certain conditions, and also several plasmatic factors of coagulation (Hjort, Rapaport, and Owren, 1955). On substances not used by the platelets, this uptake may be considered as strictly passive.

In a series of remarkable studies, Born, Ingram, and Stacey attributed this property when applied to serotonin to adenosine triphosphate (ATP). They offered the following arguments:

(*a*) The platelets are very rich in ATP (Born, 1956 [29]):

in the pig: 266 ± 14 micromoles per g of N-protein;
in the rabbit: 288 ± 51 micromoles per g of N-protein;
in man: 225 ± 23 micromoles per g of N-protein.

(*b*) 5-HT uptake by the platelets can occur in vitro in the absence of oxygen. This implies an active mechanism and therefore a source of energy which might be adenosine triphosphate (ATP).

These concentrations are only found in the adrenal medulla, in whose intracellular granules ATP is known to combine with a strong concentration of adrenalin.

(*c*) During clotting, ATP disappears from the platelets as soon as thromboplastin is formed (Born, 1956 [28]), but it does not appear in the serum as does 5-HT. It breaks down into other organic phosphorus compounds, as demonstrated by Born and Esnouf with an elegant radioactive tracer technique (1959 [34]).

This breakdown does not occur if an anticoagulant is present.

(*d*) Overload tests established a limit in the platelets' capacity to take up 5-HT; the maximal in vivo values, determined in patients with carcinoid tumors, is 3.7 molecules of ATP for 1 molecule of 5-HT. In vitro, the uptake ceases as soon as the platelets are holding 1 molecule of 5-HT for each molecule of ATP (Born, 1957 [31]). One interesting finding is that serotonin "saturation" of the platelets is not only a matter of concentration, but also of time: in vitro absorption ceases after 90 minutes (Hardisty and Stacey, 1955 [115]; Born, 1957 [31]). This interesting observation led to studying the influence of conditions in the surroundings, particularly of pH. 2,4-dinitrophenol and above all potassium distinctly increase the speed of absorption without raising its limit (Born and Gillson, 1958 [32a]). Addition of a buffer substance to correct the slight alkalinity appearing at the end of the incubation leads to the same result. These observations are compatible with the theory of an active intervention of platelet ATP as a source of energy able to modify the concentration gradient.

In a milieu containing 1 μg/ml of 5-HT, red blood cells normally free of serotonin are able to absorb it up to a concentration gradient of 2.5:1. The platelets increase plasmatic concentration to 200:1 (Stacey, 1957 [243b]). It would be interesting to investigate whether the platelets of newborn infants, which according to Mitchell and Cass (1959 [319]) are unable to take up serotonin, are endowed with a normal amount of ATP. However, the concept involving

ATP in the binding mechanism does not explain everything; besides, ATP exists in red blood cells. Reserpine, which releases 5-HT from the platelets of blocks further 5-HT. uptake, does not reduce their ATP content [29]. Some pathologic observations suggest a platelet defect in poor serotonin absorption, but no ATP intervention (Stacey [243b,] discussion p. 119). Brodie (1957 [45]) discusses 5-HT binding in cells in general, and more specifically in the platelets. He doubts the physical nature of the forces involved, which would entail speaking of adsorption rather than absorption: such a theory would be incompatible with the practically unlimited effect of a single molecule of reserpine, and with the conditioning by temperature (a Q_{10} quotient is important) of the 5-HT release and absorption mechanisms. If a chemical binding takes place, it must lead to the formation of an easily diffused, quickly dissociated compound.

C. Binding Sites. These considerations reveal a certain avidity of the circulating platelets for the serotonin contained in surrounding tissues. Since the plasma of the systemic circulation is serotonin-free—purified, as it were—this avidity can only be exerted in the immediate proximity of the sources of this substance, namely, in the blood vessels which drain the gastrointestinal tract. In fact, Toh demonstrated in 1954 [250], that the blood of the portal vein contained a greater amount of 5-HT thar. arterial blood, irrespective of the platelet count. The measurements were taken on acetone extracts from an atropinized rat colon. Hardisty and Stacey (1955 [115]) claim that fresh platelets, not yet "charged" with serotonin, effect this "charging" in immediate contact with the intestine. Udenfriend and Weissbach observed that C^{14}-labeled-5-HT had the same life span as the platelets. These two concepts are compatible: each newly formed serotonin molecule is taken up by a new platelet as soon as it enters the circulation. A fresh platelet takes charge of a fresh molecule.

(3) LIBERATION OF SEROTONIN FROM THE PLATELETS

Whatever the binding mechanism may be, it is evident that serotonin is not inactivated by amine oxidase as long as it is carried by the platelets. Under certain conditions which we are now going to examine, the platelets release an active form of 5-HT, just as they had received it.

The first of these conditions is obviously destruction of the platelets. This may occur either at the level of an injured vascular endothelium, where serotonin released from the white thrombus would induce secondary vasoconstriction, or in the spleen, thereby explaining its high serotonin content. In 1953, Cole and his collaborators [58] had made the remarkable observation that sonically disrupted platelets had no vasoconstrictive activity. We have mentioned above the pain reaction and venous constriction which may occur in the transfusion of fresh or lyophilized platelets; this reaction has been attributed to a release of 5-HT.

M. Zucker (1951 [281, 282]) holds that platelet destruction is not indispensable to the discharge of serotonin. In physiologic conditions, serotonin would be released early during clotting in vivo, under the influence of thrombin in low concentration; in vitro, Seegers' purified thrombin releases all the serotonin from the washed platelets, whether the surrounding milieu be plasma, albumin, or an isotonic salt solution (Zucker, 1955 [285]; Zucker, Lewis and Borrelli, 1956 [286]). Distilled water may be used to extract the vasoconstrictive substance from the platelets if clotting does not occur (Zucker, 1951 [281]).

Stacey revealed the influence of surfaces (1957 [243b]). The release of platelet serotonin is hastened by any physical alteration of the platelets. While this release occurs during clotting, it does not depend on it for it occurs also in afibrinogenemic plasma.

Zucker and Borrelli believe in thrombin action. According to Humphrey and Jaques (1955 [130c]), other proteolytic enzymes such as trypsin may play a part. Sarajas and his collaborators (1959 [328bis]) recognize the possibility of a massive serotonin release in extra-corporeal circuits. They blame it, as well as the ATP release from the red cells, for cardiac accidents following the use of heart-lung apparatus.

According to Toh (1956 [251]) some tissue extracts, particularly from the dog, rabbit, or sheep kidney, have the property of releasing histamine and serotonin from the platelets both in vivo and vitro. Toh suggested the name "nephrosin" for the active substance in the kidney extract. Giarman and Potter (1958 [106]) reported that brain and kidney extracts had shown high activity on the serotonin-rich tumor mastocytes of the mouse.

We cannot leave reserpine out of the list of substances able to release platelet serotonin. But this alkaloid is mainly active in blocking the uptake of serotonin by the platelets; besides, it cannot act in physiologic conditions. Reserpine action on serotonin sites, which throws a valuable light on the mechanisms, will be examined in a later chapter.

These release conditions of platelet serotonin are compatible with the concept of active binding by ATP, provided we admit at least two release mechanisms: (1) release associated with the destruction of platelet ATP through lysis or clotting; (2) when reserpine which does not affect ATP is involved, blockage of a specific binding mechanism.

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* *

Can the facts reported above be said to prove that circulating amines are inactivated by the platelets? This was suggested by Udenfriend and by Weissbach (1958 [270]). It would give the platelets a plasma-purifying function and thus complete the chemical antixenic function that they have long been known to perform on foreign bodies entering the circulation. We can accept Udenfriend's statement that any serotonin injected in excess into the venous blood is immediately taken up by the platelets. But the detoxication function is questioned by Stacey (1957 [243b], discussion p. 125-126). A computation based on the mean values of circulating serotonin (1 mg per day) and of platelet life span (at least 5 days) shows that the purifying function would affect only 0.2 mg serotonin per day. This 0.2 mg would be transformed into 5-HIA acid. Yet the daily urinary excretion of 5-HIA acid in normal subjects is 6 mg.

(4) EFFECT OF EXOGENOUS SEROTONIN ON THE NUMBER OF PLATELETS

Several experimental studies have been devoted to this subject, particularly by Bracco and Curti (1954 [38]) and by Ravetta (1955 [209]). When serotonin was injected into a rabbit in doses of 500 μg/kg, the number of circulating platelets remained unchanged during the next hour (Bounameaux and Lecomte, 1954 [292bis]). Ballerini and Cantelli (1955 [9]) injected into a rat 50 mg serotonin per kg every other day for 60 days. They observed a slight rise in the platelet count of the peripheral blood (up from 400,000 to 650,000 per mm^3) and an increase in number and activity of the bone marrow megakaryocytes. Serra and Bruni (1957 [224]) gave rats a single subcutaneous dose of 20 to 50 mg/kg and noticed a marked rise in the platelet level during the four hours after the injection. They suggested that the vasoconstrictive action of serotonin had set the clumps in motion and altered the distribution of corpuscles in the peripheral blood. Prolonged 5-HT administration to the guinea pig (40 to 320 mg per kg and per day for 20 days) caused a significant increase in the number of platelets, gradually subsiding after the treatment was stopped (Huser et al., 1959 [309bis]). In man, 5 to 8 mg of iproniazid, which blocks endogenous 5-HT breakdown and raises its threshold, will increase the number of platelets (Pletscher and Bernstein, 1958 [196b]). In some thrombocytopenia cases, Siegenthaler observed a significant increase in the number of platelets after intravenous injection in small repeated doses of 5 to 120 mg serotonin (1956 [235]).

The chief experimental results were reported by Hedinger and Langemann (1955 [121a]). In rats receiving several daily doses of 0.1 mg/kg, the platelet count remained unchanged. If the rats receive large progressive doses for two months (250 to 1,000 micromoles per kg, i.e. 44 to 170 mg/kg), the platelet count shows a marked increase, e.g., from 400,000 to 650,000 per mm^3 to 1.6 million or 1.7 million in 40 days. It must be mentioned that such high doses eventually cause renal lesions.

The rat's bleeding time, measured by immersing the tail in hot water, is markedly shortened after a single injection of serotonin (from 5 to 10 minutes down to 1 minute). In rats receiving a single 5-HT dose of 250 micromoles (44 mg) per kg of body weight, the number of platelets obtained through direct count on the tail blood is significantly high between the 8th and 12th hours (Steiner and Hedinger, 1956 [245]). This thrombocytosis coincides with eosinopenia, with the latter's lowest count during the 12th hour. Are both occurrences due to a single mechanism involving the adrenal capsules? Steiner and his collaborators (1957 [246]) tried to find the answer by operating on adrenalectomized rats. Serotonin injection still induced thrombocytosis and eosinopenia, with a slight time shift in maximal values.

(5) EFFECT OF SEROTONIN ON CLOTTING MECHANISMS IN VITRO

If 5-HT is added to the platelets of rabbit blood in a lower than 5.10^{-4} concentration, it neither induces agglutination nor alters adhesiveness (Bounameaux and Lecomte, 1954 [292bis]). In 1955, Fenichel and Seegers [90] supplied arguments supporting serotonin action in the retraction of the clot. Bovine plasma from which the platelets have been removed, and diluted 1:5 in an isotonic salt solution, shows retraction when serotonin has been added in 0.09 to 0.02% concentration. This points to a dialyzable agent in clot retraction, which would be serotonin. Ballerini (1955 [8]) believes that serotonin causes the retraction of a platelet-free clot. But in 1952, Correll [62] had rejected the action of serotonin in clot retraction. Neither Deutsch and Martiny (1958 [77]) nor Gross and Staufenberg (1958 [112]) confirmed the statements above. These authors hold that 5-HT in 0.05 to 0.1 mg/ml concentration—or 5-*dl*-hydroxytryptophan—has no influence on the retraction of a platelet-free clot. In 1959, Ballerini and Seegers [9b] admitted that the serotonin effect observed in an artificial coagulation system was not actual retraction of the clot.

A study of the action of serotonin antagonists revealed that methylserotonin in average concentration inhibited clot retraction (Deutsch and Martiny [77]). So do benzyl-l-dimethyl-2, 5-serotonin and benzyl-l-dimethyl-2,5-bufotenin. LSD 25, a potent serotonin

antagonist, and mescaline, which inhibits monoamine oxidase, have no action on clot retraction. Milne and Cohn (1957 [173]) believe serotonin to have some properties of platelet factor no. 2 (activation of fibrin formation). According to Bracco and Curti (1958 [41]), this property belongs to FC 23, a biological oxidation derivative of 5-HT.

Wiener and Udenfriend (1957 [275]) observed that serotonin had no action on in vitro coagulation (recalcification time, prothrombin level, prothrombin consumption, fibrinolysis). It does not alter the thrombelastographic picture. This was confirmed by Deutsch and Martiny (loc. cit.). These negative findings may be compared with the following observation made by Kwann and his collaborators (1957 [312a], 1958 [312b]): 5-HT would have a potent fibrinolytic action in vivo on experimentally formed thrombi in the marginal vein of the rabbit ear. Adrenalin is known to have this action, while atropine abolished the effect. In this connection, cholinergic receptors seem to play an important part in the *vasa vasorum*.

Mlle. D. Quivy (1958 [202]) found a relationship between the serotonin level and the platelet activity of serum, which might point to serotonin participation in clotting. According to Keller (1958 [138]) serotonin inhibits heparin in vitro, but only in high doses. Tryptamine has the same effect; histamine and tyramine do not.

(6) SEROTONIN IN HEMORRHAGIC DIATHESES

(*a*) Serum serotonin. Bigelow had observed that the vasoconstrictive power of serum measured on beef carotid was lower in thrombocytopenic subjects and appeared later in hemophiliacs (1953 [20]). Since then, many 5-HT assays have been done on serum and platelets in blood diseases by Bigelow (1954 [21]), Barkhan (1955 [12]), Baserga and Ballerini (1956 [14]), Hardisty and Stacey (1956 [117]), Zucker, Lewis and Borrelli (1956 [286]), Wiener and Udenfriend (1957 [275]), Polli et al. (1958 [197]), Quivy (1958 [202]). The results are so similar regardless of the method of assay that they can be given in one overall statement: a severe serotonin deficiency is to be found *in the serum* of thrombocytopenic subjects. It accompanies reduced platelet activity in the serum (Quivy [202]). It may coincide with an increased urinary excretion of 5-HIA acid (Chevallier and Fiehrer, 1956 [56]). In thrombocytopathia, Barkhan found the 5-HT content of serum normal in two cases (1955 [12]), Zucker and Borrelli found it reduced (1956 [285]), Quivy et al. found it reduced in seven patients (1958 [202]). Serum serotonin was found reduced in thrombocytoses above 500,000 platelets per mm³ (Wiener and Udenfriend, 1957 [275]) and in one case Quivy (1958 [202]). It is low in uremia patients (Zucker and Borrelli, 1956 [287]).

(*b*) Platelet serotonin content. In thrombocytopenias, the 5-HT content per platelet unit is reduced or normal. It rises only in cases of extreme deficiency, when the platelet count is lower than 100,000 per mm³ (Wiener and Udenfriend, 1957 [275]). This is not surprising since the total serotonin—even if low—is distributed among a smaller number of platelets.

The platelet serotonin content tends to decrease in severe anemias and in uremia, e.g., 0.57 μg and 0.61 μg respectively, as against 0.91-0.92 μg per billion platelets in the controls (id. [275]). The same thing happens in severe thrombocytoses. It seems to be reduced in myeloid leukemia, e.g., 0.70 ± 0.44 as against 1.22 ± 0.23 μg per billion platelets in the controls (Polli et al., 1958 [197]).

Hardisty and Stacey (1956 [117]) have made the interesting suggestion that the low serotonin content of platelets in some blood diseases might be due to a binding defect in the platelets themselves. This agrees with the fact that the platelets' ability to take up 5-HT in vitro is limited, while their ATP content seems normal.

In any case, it is generally agreed that there is no connection between the serotonin content of serum or platelets and hemorrhagic diathesis, nor between it and a symptom considered singly as bleeding time or capillary fragility.

(7) HEMOSTATIC ACTION OF SEROTONIN

The hemostatic action of serotonin is highly controversial. We shall examine it separately in animal and in man.

(a) In animals.

Bracco, Curti, and Ballerini (1954 [38a, b]) gave 300 μg serotonin in intravenous injections to guinea pigs and rats. There was a marked increase of capillary resistance measured under a cupping glass applied for 5 minutes under 500 mm mercury depression. Correale (1954 [61]) injected serotonin subcutaneously into rats; beginning with 0.4 mg/kg, the bleeding time measured on the tail was reduced. As early as 1952, Correll [62] found a hemostatic effect of serotonin on experimental wounds in rats, even after heparinization or evisceration. The effect was transient but renewable. In 1955, Conti and Ricotti [60] observed a local hemostatic action on a guinea pig after liver surgery, and Fornaroli and Koller [94] on rats with superficial wounds or visceral lesions. Lecomte and his collaborators (1954 [146]) claimed that serotonin in doses of 10 μg/kg had a favorable

action on the average bleeding time of the rabbit, either immediately or 15 minutes after the injection. 5-HT counteracts the hemorrhagic effect of isoniazid which lengthens the rabbit's average bleeding time, but does not counteract the same effect of salicylate (Estève and Laporte, 1956 [86]). Djerassi and his collaborators (1958 [78]) observed that 0.5 to 1 mg serotonin in intraperitoneal injection shortened the bleeding time in irradiated mice (750 r) and increased capillary resistance in irradiated guinea pigs (325 r).[9] These, however, were fleeting effects and required high doses in rapid administration inducing sharp dyspnea in the animals, and occasionally convulsions.

Brambel and Murphy (1959 [293]) made similar observations; 5-HT in 3 mg/kg intraperitoneal injections cut nearly in half the bleeding time of irradiated rats during the first four days after exposure to X-rays. Finally, the experiments of Niaussat and his collaborators (1958 [179a, b]) demonstrated the hemostatic action of 5-HT in local application: in unanesthetized guinea pigs, the average bleeding time of the ear was significantly shortened by application of a wet compress of a 1/5,000 dilution of 5-HT in isotonic salt solution; a compress of plain isotonic salt solution was used on the controls. Stefanini and Magalini (1956 [244]) state that 5-HT can have a dramatic effect on the bleeding time of experimental animals, while clinical results are disappointing.

(b) In man.

Intravenous or intramuscular injection of 5-HT has remarkable effect on the hemorrhages of thrombopenic purpura (Allegri and Ferrari, 1954 [1]); in repeated injections of 5 mg doses, it counteracts vascular fragility (Bracco, Curti and Ballerini, 1954 [23]). Following these reports, other publications outlined useful applications of 5-HT: prevention of bleeding during surgery (Antona, 1955 [4]); in tonsillectomy, by using 5-HT with the local anesthetic (Del Bo, 1956 [74]) and in epistaxis (Serravalli, 1955 [225]);—reduction of vascular fragility in old people (Bassani and Fantani, 1956 [15]): 2 to 4 intramuscular injections of 5 mg daily for 5 days and in tuberculous patients (Rossini, 1956 [216]).

In the treatment of thrombopenic purpura, it has been specified that 5-HT acted on vascular fragility but not on the platelet count (Ravetta, 1955 [209]). Besides, the injections must be repeated every 4 to 6 hours (Serravalli, 1955 [225], Basserga and Ballerini, 1955 [13]). Siegenthaler (1956 [235]) goes farther and says that 5-HT in intravenous drip increases the number of platelets in thrombopenic patients, while also reducing the bleeding time. The total dose may reach 120 mg but must not exceed 20 mg per hour.

Baserga and Ballerini (1955 [13]) compare the hemostatic action of serotonin and of many other amines such as sympathicomimetic amines (cf. Derouaux, 1941), stryphnone (i.e., adrenalone), and adrenochrome.

To these favorable reports Miescher (1955 [172]) opposes his own clinical experience which was entirely negative. Stefanini and Magalini (1956 [244]) deny any effect of serotonin on the bleeding time, whether in intramuscular or intravenous injections. They consider that the discrepancies between published results are due to the lack of valid tests and to the erratic character of clinical hemorrhagic symptoms. Jaques and his collaborators (1958 [135]) follow a similar conception of serotonin participation in hemostasis. Hemostasis is actually due to the combination of several factors which may occasionally compensate each other when only one is affected. If a rabbit is subjected to stress or adrenalectomy, he may survive without hemorrhagic disturbances, but his chances of survival are greatly compromised by small injections of reserpine; he then develops hemorrhages which are not due exclusively to the serotonin deficiency.

If the platelet serotonin concentration is reduced in man by prolonged intramuscular administration of reserpine (1 mg a day), neither the bleeding time, nor the clotting time, nor the capillary resistance are altered (Haverback et al., 1957 [120a]). Curti and Giuliano (1958 [66]) explained a case of hemorrhagic diathesis solely by serotonin deficiency, the platelet number and integrity remaining unchanged. In this case, serotonin had a favorable therapeutic action.

It would be optimistic indeed to claim as do Bracco and his collaborators (1957 [40]) that 5-HT administration can check hemorrhagic disturbances following large transfusions of preserved blood. Such disturbances are probably due to another cause than the rapid disappearance (within 72 hours) of serotonin from banked blood. This disappearance was also observed by Tokusawa and his collaborators (*Journal of the Japanese Society of Blood Transfusion*, 1959, 5, 240-241).

CHAPTER VIII

Action of Reserpine on Serotonin

Reserpine is an alkaloid of Rauwolfia. While not a serotonin antagonist, it has the property of displacing it from its natural sites (digestive tract, brain tissue, blood platelets). This property has been as-

[9] Bleeding time measured through sectioning the tail 1 mm from the tip; capillary resistance measured with cupping glass (12 to 15 mm mercury for 30 seconds).

serted with methods of assay and histological observations. Its interest is twofold: the artificial displacement of serotonin by reserpine is a convenient experimental procedure; besides, serotonin has been looked upon as a mediator for the action of Rauwolfia alkaloids.

1. Reserpine action on serotonin in the tissues. This action can be demonstrated by both direct and indirect arguments. In 1955, Brodie, Pletscher, and Shore [43] observed that reserpine increased the dog's urinary excretion of 5-HIA acid. In rabbits sacrificed at chosen intervals after intraperitoneal injection of 5 mg/kg reserpine, the serotonin content of the intestine decreased rapidly. The minimum—15 to 20% of the initial level—was reached 16 hours after the reserpine injection. At the 32nd hour, the serotonin level began to rise slowly again. At the same time, the serotonin content of the rabbit serum was reduced, as shown by its action on the rat uterus (Naess and Schanche, 1956 [176]). Reserpine lowers also the serotonin content of the serum, spleen, and brain in the rat and the cock (Erspamer).

Besides liberating serotonin from its sites, reserpine induces a histochemical change in the enterochromaffin cells whose granulations lose their specific coloring (Zbinden et al., 1957 [278a], in the guinea pig). According to Marks and his collaborators (1958 [162]) who have made a careful check of colorable granulations, chlorpromazine has no such cytological effect. Treatment by iproniazid (Marsilid, or 2-isopropylhydrazide of isonicotinic acid) reduces the loss of histochemical activity in enterochromaffin cells; it also minimizes the reduction of the total serotonin content in the intestine evoked by reserpine (Zbinden et al., id.). Reserpine causes serotonin to disappear from the skin gland granulations of the toad *(Discoglossus pictus)* and the frog *(Rana temporaria)* (Liebecq-Hutter and Bacq, 1958 [156]).

Under reserpine action, Pletscher (1957 [196a]) was able to distinguish two functionally different regions in the rabbit stomach: the cells of the prepyloric region, similar in structure to the small intestine, become poorer in 5-HT as they lose their argentaffine properties; the fundus cells, which alone have an acid secretion, retain their 5-HT and their color characteristics.

The number of platelets does not change, nor does the bleeding time. Haverback (1957 [120a])* says that the daily administration of small doses to man practically depletes the platelets of serotonin (less than 10% of their normal content); the bleeding time, clotting time, prothrombin level, prothrombin consumption and clot retraction time remain unchanged. Platelet serotonin content in man decreases for two days after a single intramuscular injection of reserpine (1 mg); it does not become normal again until three weeks later (Hardisty, Ingram and Stacey, 1956 [116]). Haverback and his collaborators have observed the cumulative effect of small reserpine doses in man (1956 [118]): a single dose of 1 mg has no action; after ten daily doses of 1 mg, the platelet serotonin content drops by 95%. The same depletion occurs in the rabbit with reserpine doses of 0.015 mg/kg. In the guinea pig, 0.015 mg/kg doses deplete the platelets of serotonin within a week; yet the serotonin level in the brain is only 30% reduced (Haverback et al., 1957 [120a]).

Reserpine enters the brain tissue quickly; in the rabbit, the saturation point is reached within 10 to 15 minutes. It then disappears completely in 8 hours. Reserpine is rapidly eliminated from the body (Hardisty et al., 1956 [116]), yet its sedative effects last 48 hours (Hoagland, 1957 [126]).

Reserpine effects in vivo are thus both cumulative and lasting. Brodie even called them 'irreversible" [45].

2. Reserpine action on platelet serotonin. This action has been studied with special interest. Shore, Brodie et al. observed in 1956 [233] that reserpine administered to the rabbit, rat, or guinea pig induced serotonin release from the platelets without destroying them. Spectrofluorophotometry was used for the 5-HT assay (Udenfriend's technique, 1955). In rabbits given 5 mg/kg reserpine in intravenous injections, whole blood serotonin drops from 3.6 to 0.15 mg/ml in 16 to 48 hours; return to the normal level takes 6 days. In rats, the serotonin level declined from 0.1-0.2 μg per mg protein to 0.008 μg; in guinea pigs, from 0.4-0.5 μg to 0.02-0.04 μg per mg protein.

Reserpine releases serotonin from the platelets in vivo and has the same action in vitro (Carlsson, Shore, Brodie, 1957 [53]). Temperature is an essential condition; the release is maximum at +37°C, and negligible at +2°C.

Measurable amounts of 5-HT are released by reserpine above a very low threshold of 0.05 μg/ml (i.e., 10^{-7} M); the maximum is reached at 0.3 μg/ml and is never exceeded, no matter how high the concentration of reserpine.

The two observations above seem to point to chemical rather than physical binding forces (cf. Brodie, 1957 [45]).

One molecule of reserpine or of any other tranquilizing alkaloid of Rauwolfia releases hundreds of serotonin molecules. This is fortunate for the investigator. It enables him to observe what happens after the platelets have been depleted of their serotonin, as well as the conditions of binding and release of serotonin by the platelets.

3. Release mechanism. Reserpine releases plate-

* Reference given as "in [154a]" in the French text. Checked and corrected to [120a] by the translator.

let serotonin in vivo and in vitro. We have already mentioned that it did not antagonize serotonin in vitro (Naess and Schanche, 1956 [176]). We must also mention that reserpine does not block the biosynthesis of 5-HT. Erspamer and Ciceri made this observation in dogs and rats in 1957 [85a]. Nor has it any action on breakdown metabolism, since the urinary execretion of 5-HIA acid remains unchanged in patients given reserpine treatment for carcinoid tumors (Haverback et al., 1956 [119]). The only logical conclusion is that serotonin is still being formed but no longer taken up. In rabbits given reserpine (5 mg/kg) 16 hours before the drawing of blood, the platelets show poor ability to take up serotonin in vitro (Kuntzman et al., 1956 [41]). We may therefore consider that reserpine blocks the binding of serotonin * in its preferred sites in the body, i.e., in the platelets and accessorily the brain tissue (Haverback et al., 1956 [118]); this blocking action is cumulative. But we have seen that there were at least two binding mechanisms of serotonin in the platelets; one of these involves ATP (Born et al.) on which reserpine has no action (Born et al., 1958 [30]). A second active mechanism, as yet little known, must therefore be concerned. In any case, under the influence of reserpine the platelets have become somehow "indifferent" to serotonin. Like bufotenine, serotonin undergoes a passive diffusion which considerably lowers its concentration (Hughes et al., 1958 [129]).

Reserpine is not the only Rauwolfia alkaloid able to release platelet serotonin. Moreover, this action seems due to a metabolite rather than to reserpine itself. A comprehensive inventory was taken in order to determine the responsible chemical group; only pharmacologically active alkaloids, i.e., with tranquilizing effects (reserpine, rescinnamine, deserpidine, raunsescine, 11-desmethoxy reserpine, syrosingopine **) are able to displace serotonin either from the brain sites (Brodie et al., 1956 [44]) or from the platelets (Shore et al., 1956 [232]). The same observation was made in vitro on platelet serotonin (Carlsson et al. [53]). But the active chemical group has yet to be determined. This shows clearly that the same binding mechanism works in the platelets and the brain cells, in spite of very different histological structures.

The effects of reserpine on the brain cannot be well understood unless serotonin affinity is recognized to be somewhat selective. Cronheim and Gourzin (1956 [65]) think that reserpine effects an elective blocking of 5-HT access to its sites; serotonin keeps forming at the usual rate and finds sites it does not normally reach, such as subcortical sites.

Finally, we will mention for information that reserpine has been said to have a favorable action on mouse leukemia, which serotonin alone does not have (Goldin et al., 1957 [108]).

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* *

Delay and his collaborators (1959 [73b]) have recently announced that psilocybin, an indole structure drug isolated from Mexican hallucinogenic mushrooms, was able to displace serotonin, as is reserpine. The urinary excretion of breakdown products, especially of 5-HIA acid, was found considerably increased in man and rabbit.

CHAPTER IX

Serotonin in Pathology
(exclusive of hemorrhagic diatheses)

1. SEROTONIN IN VASCULAR HYPERTENSION

The transient hypertensive effect of 5-HT observed in animal experiments has sometimes been thought to show that serotonin was a determining factor in human vascular hypertension. This hypothesis was contradicted by the mediator role of serotonin in hypertensive subjects under reserpine treatment. As early as 1954, Erspamer said that the serum 5-HT content of hypertensive subjects was never above, and sometimes below normal (cf. [84]). This statement has been confirmed many times, particularly by Durlach (1957 [80]). Poumailloux and Labignette (1959 [199a]) assayed 5-HIA acid in patients' urine and compared the values found with normal ones of 2 to 4 μg per ml, or 2 to 6 mg in 24 hours; urinary excretion of the metabolite showed no significant change in hypertensive patients. Few new facts have come up on this subject. Urinary excretion of 5-HIA acid declined in 3 out of 6 hypertensive patients with kidney involvement (Borges and Bessman, 1956 [27]). 5-HT induced hypotension in ¼ of the patients treated for essential hypertension (Wilkins and Hollander, 1957 [276b]). Gallini's review (1957 [99]) mentioned several hypotheses but no experimental facts.

Durlach presented a somewhat "rejuvenated" concept of serotonin participation in vascular hypertension (1957 [80]). Hypertensive disease is not due to pressor substances in excess, but to a chronic deficiency of methylserotonines. This concept is based on the antiserotonin action of some hypotensive agents used

* Reserpine in the French text. Translator's correction.

** Fr. méthyl-réserpate-syringate. Translator's note.

in therapeutics, such as hydrazinophtalazine and reserpine; some serotonin antimetabolites, particularly 2,5-dimethyl-serotonin and BAS, have hypotensive effects (cf. Woolley and Shaw). Clinical experiments with BAS have been encouraging in spite of untoward effects similar to those of reserpine: nasal congestion, intestinal cramps, and drowsiness (Wilkins, 1956 [276a]).

2. SEROTONIN AND ANAPHYLAXIS

We have purposely brought together the study of capillary permeability and anaphylaxis in connection with serotonin because the two problems are closely interrelated.

(*a*) Serotonin and capillary permeability. Does 5-HT have an autonomous action on capillary permeability? According to Feldberg and Smith (1953), 5-HT is able to release histamine locally. Binet and Burstein (loc. cit.) find in this mechanism the explanation of the vasodilation of the 5-HT-perfused dog leg. Binet and Quivy (1958 [23b]), seeking confirmation of this, injected 5-HT into a rat leg and observed a significant depletion of histamine (as much as 42.4%) in the skin tissues of the leg. They further found (1958 [23c]) that this histamine release was unaffected by preliminary administration of sodium salicylate, which is sometimes said to have an anti-anaphylactic action. Kelemen (1958 [310]) had reached the opposite conclusion that subcutaneous administration of sodium salicylate to the adult rat (600 mg/kg) inhibited the development of edema in the hind-paw after a 2.5 μg injection of 5-HT.

The histamine-releasing effect of 5-HT has also been studied by Georges and his collaborators (1956, 1958). Peritoneal administration of 5-HT to the rat 30 minutes before injection of dextran under the skin of the sole significantly reduces the edema reaction. But if 5-HT is injected into the sole of the rat paw, it induces an edema similar to that induced by dextran or albumin (Benditt et al., 1955, quoted by Georges et al. [102]). On the other hand, intraperitoneal injection of 5-HT prevents the edema induced by the local injection of 5-HT (Georges et al., 1958 [102]). Preliminary myelotomy practiced on the rat markedly increases the inflammatory edema induced by 5-HT.

Rabbit plasmas in which antigen-antibody reactions occurred induce an increase of capillary permeability when injected subcutaneously to rabbits, whether platelets are present or not (Humphrey and Jaques, 1955 [130c]). The question is whether this capillary action is due to histamine or to an indirect effect of 5-HT. Perfusion of various histamine-releasing substances into the rat hind part (dextran 1935 L and 48/80) causes sizable amounts of histamine and serotonin to appear in the venous blood. This serotonin comes from the perfused tissues since the rat skin has lost nearly half its 5-HT content (measured by Erspamer's technique on the rat uterus) (Halpern et al., 1957 [155]).

It would be a gross over-simplification to assume that 5-HT effect on capillary permeability is due solely to an indirect action on histamine. The edematigenous effect of 5-HT—increase of local capillary permeability—is higher than that of histamine. Specific antagonists differentiate the two: dibenamine antagonizes 5-HT, pyrilamine antagonizes histamine (Rowley and Benditt, 1956 [217]). On the rat capillaries, 5-HT is about 30 times more active than histamine; it is much less active on the guinea pig capillaries (Liacopoulos et al., 1957 [155]). LSD 25 is a specific inhibitor of 5-HT action on capillary permeability. Promethazine, a potent histamine-antagonist, and chlorpromazine, a weak one, both antagonize 5-HT.

In the rat, the increase of capillary permeability induced by intradermic 5-HT injection is strongly inhibited by chlorpromazine, BOL 148, sodium salicylate, and less markedly by nivaquine. No inhibitor action is exerted by ACTH, cortisone or hydrocortisone, anti-inflammation corticosteroids, somatotropin, butazolidin, and promethazine (Van Cauwenberghe et al., 1958 [263a]; 1959 [263b]). To investigate capillary permeability, these authors gave rats an intraperitoneal injection of chlorazol blue sky at 1% (10 ml/100 g); extravasation showed more or less rapidly as a blue coloring in the areas of intradermic injections of 5-HT (100 μg 5-HT in 0.1 ml isotonic solution).

Spector and Willoughby (1957 [240a]) mention the high 5-HT content of some traumatic exudates induced in the rat. They believe that 5-HT increases capillary permeability in a 1 to 10 μg/ml dose, while having a vasoconstrictor effect at 100 μg/ml and above. The 5-HT released by inflamed tissues may have a direct action on capillary permeability.

According to Benda (1957 [17c]), preliminary intraperitoneal injection of normal human serum reduces the plantar edema induced by serotonin in the rat. The reduction is more marked with serum from a psychotic subject.

(*b*) Serotonin and anaphylactic shock. It has been known since Rocha and Silva's experiments that histamine could not alone account for all the symptoms of anaphylactic shock. Does serotonin participate in shock mechanisms?

(1) This participation has sometimes been inferred from serotonin's ability to elicit at least some of the symptoms of shock. West and Parratt (1957, [274]) state that serotonin is necessary to cause the rat leg edema, which is an anaphylactoid reaction. No edema

response is elicited in the rat under reserpine treatment which depletes its skin of serotonin. On the other hand polymyxin B, which displaces histamine from the rat skin, does not inhibit the edema reaction. When injected subcutaneously into the back of the rat leg, 5-HT and histamine induce roughly the same edema lesion without damaging the mastocytes (Rowley and Benditt, 1956 [217]).

Some antihistaminics used in the clinical treatment of allergic reactions have in addition some antiserotonin properties. Two phenothiazines (promethazine and chlorpromazine), and to a lesser degree two piperidines (thenalidine and 4-N-benzyl-anilinomethyl-piperidine) inhibit 5HT action on the isolated rat uterus in vitro, and the 5-HT induced edema of the rat leg in vivo (Doepfner and Cerletti, 1959 [252]).

Guinea pigs exposed to a 1% 5-HT aerosol in a closed space present within 60 to 150 seconds a shock syndrome similar to that induced by histamine and acetylcholine under the same administration conditions, as well as by the antigen to which the animals have been sensitized (Herxheimer [123]). The shock syndrome is completely inhibited by LSD 25, a well-known serotonin-antagonist; it is partially inhibited by atropine (Herxheimer, 1955 [123]), chlorpromazine and thenalidine (Benda and Miravet, 1957 [17a]; Konzett, in [252]). It is seldom lethal; repetition with increasing doses brings tachyphylaxy.

(2) Histamine and serotonin are released from the rabbit platelets in plasmatic milieu in vitro by the antigen-antibody reaction (Humphrey and Jaques [130c]). The same observation was made on the rabbit in vivo by Waalkes and his collaborators (1957 [265, 266]).

According to Weissbach (Ph. D. thesis, 1957 [268]), thrombopenia which is always present in the anaphylactic shock is accompanied by a drop in the histamine and serotonin level of the whole blood. But free amines keep rising in the plasma, specifically serotonin supplied by the gastrointestinal tract, lungs, and nerve tissue. These free amines are no longer inactivated by the platelets and are therefore in relative excess.

Fink (1956 [91], 1958 [92]) found the egg white-sensitized mouse uterus 1,000 times more sensitive to serotonin than to histamine. This sensitivity was totally abolished by addition of LSD 25 or by a reserpine treatment. Considering what a huge amount of histamine would be needed to elicit an anaphylactic reaction in the mouse, this reaction is more likely to depend on the release of serotonin.

(3) The rabbit's urinary excretion of 5-HIA acid is increased during anaphylactic shock (Fischer and Lecomte, 1956 [93]).

The above three sets of arguments may have seemed convincing; yet they call for a critical examination. Special consideration must be given to the widely varied symptoms of anaphylactic shock and to the uneven distribution of histamine and serotonin among animal species.

The rabbit's platelets are the only ones to contain histamine in significant amounts, while the platelets of all mammals contain serotonin in varying amounts. The high serotonin content of the rat skin is a striking exception (West). Sanyal and West (1958 [221]) consider that 5-HT plays a minor part in the guinea pig anaphylaxy. The dog's anaphylactic reaction releases histamine, but no serotonin. The symptoms of anaphylactic shock in the rat have provided no evidence of 5-HT or even histamine participation. The shock occurs in animals whose skin and intestine have been "purged" of these amines, as well as under the action of their specific antagonists. Medakovic and Spuzic (1959 [170b]) observed a high 5-HT level in the blood of the adrenalectomized rat subjected to an experimental traumatic shock through ischemia; yet they were unable to establish a cause-and-effect relationship between one and the other. Garcia Arocha (1959 [304]) observed in vitro reactions of the sensitized female rat organs to antigen addition. He concluded that the anaphylactic response of the rat uterus was dependent on the release of 5-HT. In other tissues, both histamine and 5-HT participate in this response.

The accumulation of histamine and 5-HT in the rabbit lung after the anaphylactic shock may be due to the platelets being held in the lung in connection with the antigen-antibody reaction (Schachter, 1953 [329]).

On our part, we mentioned (specifically in the thesis of our student Lemoine, Paris 1957) that human platelets tagged with a radioactive tracer were massively stopped by the rabbit lung if the rabbit had first been sensitized. We did not make an assay of 5-HT. The guinea pig lung is very rich in histamine and poor in serotonin; the reverse is found in the mouse lung. The rat and rabbit lung contain both histamine and serotonin (Weissbach et al., 1957 [269]). It is obviously difficult to extend the conclusions of experimental results from one species to the next. The release of serotonin of the rabbit plasma during anaphylactic shock may be simply a side phenomenon. In reserpine-treated sensitized rabbits, shock is accompanied by a release of histamine in the plasma, but not of 5-HT (Schachter, 1953 [329], Waalkes et al., 1957 [265 and 266]).

More positive objections to a major role of serotonin in anaphylaxy will now be given. Lecomte and Van de Berg (1958 [148a]) underline the marked difference between the cutaneous vasomotor reactions of the rabbit to serotonin and anaphylactic symptoms.

The former are immediate reduction of skin vascular activity, corresponding with general hypotension (3 to 4 minutes for 250 μg/kg) and local hypothermia, followed by vasodilation. According to Inoue and Kuriaki (1957 [131], the 5-HT content of guinea pig plasma does not change during anaphylactic shock. BOL 148, a 5-HT antagonist, does not inhibit anaphylactic shock in cocks sensitized with pig serum when the releasing injection is given (Lecomte and Beaumariage, 1957 [147]).

A few more facts should be mentioned in connection with serotonin action in anaphylactic shock. Lecomte and Fischer (1958 [148b]) state that urinary excretion of 5-HIA acid is not affected in man by the rapid intravenous injection of a histamine releaser. Parrot and Mlle. Flavian (1959 [324]) found that normal human serum could take up 5-HT in vitro, while the serum of allergic subjects could not. The whooping cough bacillus *(Hemophilus pertussis)* gives to the mouse an amazing sensitivity to the action of serotonin (Munoz, 1957 [175]). This very specific sensitivity extends to some endotoxins *(Escherichia coli, Serratia marcescens)* (Kind, 1959 [139]). Gilbert (1959 [305]) believes that histamine and serotonin have a part in the initial response of the anesthetized cat which is given 0.05 mg/kg endotoxine E in intravenous injection. According to Davis and his collaborators (1959 [299]), intravenous injection of *Escherichia coli* endotoxine rapidly induces thrombopenia; it is soon followed by a fall in the 5-HT level of the peripheral circulation blood. 5-HT concentration in the pulmonary vascular bed shows a sharp transient rise.

Finally, Herxheimer (1955 [123], 1957 [124]) provided a weighty argument against serotonin participation in the guinea pig symptoms of anaphylactic shock. LSD 25, a choice serotonin-antagonist, has no protective effect when administered before the releasing injection. Yet the same LSD 25 protects the guinea pig against the shock induced by 5-HT in aerosol administration.

Serotonin and carcinoids

The literature concerning the malignant carcinoid syndrome and its relations with serotonin has been reviewed by Lembeck (1956 [149]) and recently by Peskin and Orloff (1959 [191]), Hedinger (1959 [121b]), and Séhabiague (Thesis, Bordeaux 1959 [332]).

We are borrowing from Peskin and Orloff the main part of their excellent historic review.

Carcinoid tumors of the intestine were first described by Lubarsch 70 years ago. They were long believed to be benign, until about 1930 when Cassidy observed widespread metastases and stenosis of the pulmonary valves. The relationship, first between the various clinical symptoms, and later with serotonin was definitely established almost simultaneously in Sweden in 1954 by Thorson and his collaborators, and in 1953 in Switzerland by Isler and Hedinger and in the United States by Rosenbaum and his collaborators.

Erspamer and Rapport, followed by Barter and Pearse (1953-1955) and by Gomori (1954) confirmed the presence of 5-HT in normal argentaffin, or Kultschitzky cells. The vasoconstrictive substance extracted from carcinoid tumors in 1936 was identified as 5-HT by Erspamer and Asero in 1952, and by Ratzenhofer and Lembeck in 1954.

These positive findings, added to the fact that no primitive carcinoid was ever found in tissues free of argentaffin cells, have supplied the histological and even endocrinological explanation of the problem. The Kultschitzky cells, which have a high decarboxylase content, produce 5-HT and release it into the blood. Record 5-HT values were found in assays made on tumor tissues: 800 μg/g (Sjoerdsma et al. [237]), 2800 μg/g (Lemberg [149]), 100 to 800 μg/g (Zylka and Köhler, 1957 [290]). The most frequent anatomical origin is the appendix or the small intestine.

Sjoerdsma, Weissbach and Udenfriend (1956 [237]) have outlined some of the biological and biochemical clinical elements of the malignant carcinoid syndrome (cf. chart published in the *A.M.A. Archives of Internal Medicine,* 1957, 99, 1009-1012; also Schneckloth et al., *Circulation,* 1959, 19, 766-772).

(*a*) "Flushing" is a major clinical symptom. Thorson describes three phases in this peripheral vasodilation: (1) facial redness and burning spots spreading to the body and extremities; (2) generalized redness caused by over-all dilation of the teguments, with accelerated pulse and high blood pressure (central vasoconstriction); (3) cyanosis, sometimes with bradycardia and hypotension. Flushing attacks are repeated at irregular intervals; they are made more severe or frequent by the ingestion of alcoholic or hot drinks, by the massage of the tumor, and by the administration of iproniazid (Kabakow et al., 1958 [136]). They may be accompanied by explosive defecation and by anxiety, even angor. In chronic conditions, flushing appears as persisting cyanosis with teleangiectasis. The malignant syndrome is completed by chronic diarrhea, respiratory disturbances, valvular lesions especially in the right heart, reduced renal functioning, and sometimes arthritis.

(*b*) Biological syndrome.—Laboratory tests reveal an increased urinary excretion of 5-HIA acid (Heilmeyer et al., 1956 [122]). The normal rate of 2 to 9 mg/24 hrs. (unless bananas were eaten) is considerably higher and may reach 76 to 580 mg per 24 hours (Sjoerdsma et al., 1957 [237]). Lembeck gives the normal urinary 5-HIA acid values as 1.1 to 5.1 μg per ml, increasing to hundreds of μg, up to 2 mg per ml in

carcinoid tumor patients.

Urinary excretion of 5-HIA acid is increased by reserpine and reduced by chlorpromazine (Peskin and Orloff, loc. cit.). Unmetabolized 5-HT may even be found in urine which normally shows only traces of it. The serum serotonin level is very high; instead of the normal 0.1 μg,* it goes up to 2 μg/ml (Zylka and Köhler [290]), 0.5 to 2.7 μg (Sjoerdsma et al., 1956 [237]), 1 to 5 μg (Lembeck, 1956). An increase in the blood serotonin level was considered an exception by Schmid and his collaborators: 1 out of 31 cases (1959 [329 bis]). Sandler and Snow (1958 [220b]) have described a few cases of carcinoid tumors with clinical variations; they secreted 5-hydroxytryptophan instead of 5-HT. Dengler (Klinische Wochenschrift, 1959, 37, 1245-1248) observed an atypical tumor of the pancreas with massive urinary excretion of 5-HIA acid.

(*c*) Biochemical syndrome.—In balance studies, the use of C^{14}-labelled tryptophan has clearly revealed the precursor role of dietary tryptophan; its metabolism is considerably altered in carcinoid tumor patients, in whom 60% of the ingested tryptophan—instead of the normal 1%—are transformed into hydroxyindoles.

Physiopathology. Most of the anatomical and clinical elements seem related to a massive release of endogenous serotonin. Valvular lesions are difficult to explain satisfactorily. On corpses, Zylka and Köhler (1957) observed a significant difference in the blood serotonin content of the right heart (0.4 μg per ml) and the left heart (only 0.15 μg per ml).

A survey of recent literature by Beerman and Pastras (1959 [16]) specifies some interesting points. The tricuspid and pulmonary valves are the only ones affected. "These valves become vascularized, thickened, sclerotic and deformed. The sclerotic tissue may extend and form well-defined cushions overlying the intima of the pulmonary artery or the endocardium of the right atrium. It may extend to involve the thickened and fused cordae of the tricuspid valve."** Spain (quoted by [16]) ascribes these impairments to an actual dietary deficiency in tryptophan, since the metabolism is altered and a large amount of tryptophan is used by the tumor. Bantus have been observed to show frequent endocardial fibrosis in relation with a tryptophan-deficient diet.

Therapeutics. Attempts to use serotonin antagonists to fight the major symptoms of carcinoid tumors have been disappointing. While there is positive evidence of excessive circulating serotonin, no pharmacological product can reduce it without risks. Treatment will therefore be essentially surgical.

* 6.1 μg in the French text. Checked and corrected by the translator.

** Beerman and Pastras [16]. Translator's note.

CONCLUSION

Serotonin is widely distributed in nature among the animal and vegetable kingdoms. Its distribution in the organism of man and mammals is somewhat puzzling. Serotonin is formed mainly by the argentaffin cells of the gastrointestinal tract and carried by the blood platelets; the central nervous system is one of its sites.

The main secrets of the biological synthesis and breakdown of this indolealkylamine were uncovered by chemists. Within a few years, serotonin has become one of the substances most frequently, if not most successfully studied by pharmacologists, at least for its basic action on smooth muscle. Driven out of its haunts by reserpine, stripped of its activity by its antimetabolites, serotonin has been given a merciless chase. But we are yet far from able to define its biological role. Various hypotheses have described it as (1) an antidiuretic hormone (Erspamer), (2) a blood pressure regulator (Corcoran), (3) a hemostatic agent (Bracco and Curti), (4) an anaphylactic shock agent (Weissbach), (5) a chemical mediator (Brodie, Welsh), and (6) a growth factor (Laborit, Niaussat). Serotonin has given no evidence of any of these roles, except that of chemical mediator. At most, we can recognize in the malignant carcinoid syndrome a specific fate of the chemical molecule which might in some ways imply a hormone. The 5-HT secreted in the blood in large amounts by the intestinal tumor exerts its action in remote parts of the organism. But the same action might be due to a waste substance; with carcinoid tumors we enter a truly pathological realm. Whether serotonin is able under normal conditions to act as a real hormone in the functioning of some organ has yet to be demonstrated.

Actually, it matters little whether the hypotheses mentioned were true or false. Formulating them has had the valuable effect of inspiring a great deal of research; if no therapeutic applications have resulted, the stirring of concepts is in itself fruitful.

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Notes and News

Pilgrims Land Twice In New York

"Pilgrim's Progress," a series of two excellent articles about Pilgrim State Hospital, appeared in September 30 and October 7 issues of the *New Yorker*. The articles describe the work of Dr. Henry Brill and the staff of the Hospital, with emphasis on the changes accompanying the use of psychotropic drugs.

Office of Technical Services Offers Bibliography

The Office of Technical Services (U. S. Dept. of Commerce) recently issued a 12-page selective bibliography of "Tranquilizers and Stimulants." This is a bibliography of reports listed in the two OTS monthly abstract journals: *U. S. Government Reports* and *Technical Translations.*

Twenty-two U. S. Government research reports published between 1948 and 1959 are listed in the bibliography, along with 39 translations of articles and monographs which appeared in foreign journals: Russian 15, German 10, French 8, Italian 4, Czech 1, and Spanish 1. Approximately three-fourths of the entries are accompanied by a short abstract.

The bibliography (report number SB-433) may be obtained for 10 cents by writing to the Office of Technical Services, U. S. Dept. of Commerce, Washington 25, D. C.

Drug Manufacturers Look Into Crystal Ball

The *American Druggist* annually asks pharmaceutical manufacturers to predict the products they expect "in the next five to ten years." A recent tabulation of the results of the 1961 predictions showed that for the fourth successive year the type of product mentioned most often was "drugs which stimulate, depress, or in other ways affect the central nervous system and permit therapeutic approaches to mental illness." (Source: *New Medical Materia*, August 1961)

New Phenothiazine Drug Proves Potent Coronary Vasodilator

The main activity of a new phenothiazine derivative is its effect on the cardiovascular system, according to the results of experimental pharmacologic investigations by V. V. Sakusov and his associates.* The compound, synthesized at the Institute of Pharmacology and Chemotherapy of the Academy of Medical Sciences of the U.S.S.R., is chloracyzin: 2-chloro-10 (B-diethylaminopropionyl) phenothiazine hydrochloride.

Sakusov reports that chloracyzin exhibits an antihistaminic effect and anticholinergic action, being about one-half to one-fourth as active as atropine. The drug has a marked effect on coronary circulation, increasing the outflow of blood from the coronary sinus of the cat by 80 to 100% of the original volume. However, no change in the blood pressure was observed, and the drug apparently does not affect the peripheral blood vessels. Chloracyzin is also effective after experimental spasm of the heart vessels has been induced by pituitrin.

From extensive animal experiments, it was concluded that chloracyzin has the power to cause considerable dilation of the cardiac vessels, but does not affect the blood pressure. Results of preliminary clinical studies in man show that this drug may be effective in the treatment of angina pectoris.

Special Award of $5,000 Goes to Dr. Felix

Dr. R. H. Felix, Director of the National Institute of Mental Health, received on December 7 a Rockefeller Public Service Award of $5,000 for his "distinguished contributions to the field of science." The award also permits Dr. Felix to draw upon additional grant money "to allow the fulfillment of the winner's project."

* Academy of Medical Sciences of the U.S.S.R., Institute of Pharmacology and Chemotherapy. Scientific Papers Vol. II. New Data on the Pharmacology of the Coronary Circulation. V. V. Sakusov, Ed. In Russian with English summaries. Vikhlaev, Yu. I., Kaverine, N. V., The Pharmacology Chloracyzin, *Pharmacology and Toxicology*, 1959, 22:24-29.

Recent NIMH Grants

Between May 1, 1961, and October 1, 1961, the National Institute of Mental Health awarded the following grants in support of research relevant to psychopharmacology. (The July 1961 PSC *Bulletin* described the NIMH-supported projects in this field that were active as of May 1.) Requests for detailed information about the research should be addressed to the principal investigator of the project.

Adler, M. W., Temple University, Philadelphia, Pennsylvania. Chronic Brain Lesions and Responsiveness to Drugs.

Barmack, J. E., Public Service Research Institute, Stamford, Connecticut. Performance after Ingestion of Depressant Drugs.

Bockoven, J. S. et al., Cushing Hospital, Framingham, Massachusetts. Drug Effects on Behavior and Cognition in the Aged.

Bradley, P. B., Birmingham University, Birmingham, England. A Neuropharmacological Study of Cerebral Function.

Brill, H., Research Foundation for Mental Hygiene, Inc., Department of Mental Hygiene, Albany, New York. NIMH Collaborative Study.

Carver, M. J., University of Nebraska College of Medicine, Omaha, Nebraska. Amino Acid Transport by White Blood Cells.

Denber, H. C. B., Manhattan State Hospital, Ward's Island, New York, New York. Polygraph Studies with Mescaline.

Deneau, G. A., and Seevers, M. H., University of Michigan Medical School, Ann Arbor, Michigan. Dependence on Narcotics in Primates.

Dinsmoor, J. A., Indiana University, Bloomington, Indiana. Action of Drugs on Behavior Suppressed by Punishment.

Ferris, J. P., Florida State University, Tallahassee, Florida. The Constitution of Some Lythraceae Alkaloids.

Fink, M., Hillside Hospital, Glen Oaks, Long Island, New York. Conference on "EEG and Human Psychopharmacology."

Fischer, G. J., and Davis, W. M., University of Oklahoma, Norman, Oklahoma. Physiological Factors in Imprintability.

Flach, F. F., and Burrell, C. D., Cornell University Medical School, New York. Effect of Imipramine and ECT on Calcium in Depression.

Grossman, S. P., State University of Iowa, Iowa City, Iowa. Behavioral Effect of Local Chemical Brain Stimulation.

Gutekunst, R. M., Jr., and Youniss, J. E., The Catholic University of America, Washington, D. C. Imprinting and a Test of the Consolidation Hypothesis.

Guth, P. S., Tulane University School of Medicine, New Orleans, Louisiana. Interaction of Neurohumors and LSD Congeners in CNS.

Hollister, L. E., and Overall, J. E., Veterans Administration Hospital, Palo Alto, California, and Veterans Administration Central Neuropsychiatric Research Laboratory, Perry Point, Maryland. Cooperative Clinical Screening of Psychotropic Drugs.

Holmberg, K. G., Centrallasarettet, Danderyd, Sweden, Autonomic Drug Effects in Varying Emotional States.

Hudson, R. D., University of Michigan Medical School, Ann Arbor, Michigan. Drug Effects on Pyramidal and Extrapyramidal Systems.

Issac, W., Emory University, Atlanta, Georgia. Quantification of Arousal and Arousal Stimuli.

Jarvik, M. E., Albert Einstein College of Medicine, Yeshiva University, New York, New York. Effects of Drugs upon Memory.

Kaplan, S. D., et al., Lincoln State Hospital, Lincoln, Nebraska. Visual-Olfactory vs. Vascular Schizokinesis.

Klein, D. F., and Fink, M., Hillside Hospital, Glen Oaks, Long Island, New York. Psychiatric Reaction Patterns to Psychotropic Drugs.

Koella, W. P., and Bergen, J. R., Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts. Action of Plasma Proteins and Psychotogens.

Kurland, A. A., and Hanlon, T. E., Spring Grove State Hospital, Baltimore, Maryland. Method Validation in the Follow-Up of Drug Treatment.

Leevy, C. M., and Nenno, R. P., Seton Hall College of Medicine, Jersey City, New Jersey. Biochemical Mechanism of Delirium Tremens.

Lindberg, T. F., et al., Medfield State Hospital, Harding, Massachusetts. Drug Therapy for Depressed and Schizoid Alcoholics.

Lubin, A., and Lana, R. E., The American University, Washington, D. C. Repeated Measurement Design in Psychopharmacology.

Maher, B. A., Harvard University, Cambridge, Massachusetts. Telemetric Autonomic Measure of Conflict Behavior.

Moraczewski, A. S., Texas Medical Center Houston, Texas. Pharmacological Differences among Brain Mitochondria.

Nelson, G. H., New Castle State Hospital, New Castle, Indiana. Effects of Marsilid on Ethanol Consumption.

Prange, A. J., Jr., and Lipton, M. A., University of North Carolina, Chapel Hill, North Carolina. Criteria for the Effective Treatment of Depression.

Rowland, V., et al., Western Reserve University, Cleveland, Ohio. Electrophysiology and Pharmacology of Conditioning.

Sam, J., The University of Mississippi, University, Mississippi. Synthetic Relatives of Noscapine.

Schachter, S., Columbia University, New York, New York. Cognitive and Physiological Determinants of Emotion.

Shapira, J., and Perkins, W. H., University of Arkansas Medical Center, Little Rock, Arkansas. Neutron Activation Analysis of Trace Metals.

Sidman, M., Massachusetts General Hospital, Boston, Massachusetts. Sensory and Behavioral Tests of Induced CNS Changes.

Smith, K. U., and Shideman, F. E., University of Wisconsin, Madison, Wisconsin. Drugs and Brain Biochemistry in Aging and Development.

Toman, J. E. P., and Sabelli, H. C., Chicago Medical School, Chicago, Illinois. Central Receptor Sites for Psychotropic Drugs.

Vaillant, G. E., Massachusetts Mental Health Center, Boston, Massachusetts. The Estimation of Chlorpromazine in Human CSF.

van Breemen, V. L., The Mercy Institute for Biomedical Research, Denver, Colorado. Electron Microscopy of Effects of Drugs on Neurons.

Weiner, H., Saint Elizabeths Hospital, Washington, D. C. The Effects of Drugs on Sensori-Perceptual Processes.

Weitzman, E. D., Albert Einstein College of Medicine, New York, New York. A Behavioral Method for the Study of Pain in Monkeys.

Welt, I. D., Institute for the Advancement of Medical Communication, Washington, D. C. Indexing of World Literature in Psychopharmacology.

Wilcott, R. C., Western Reserve University, Cleveland, Ohio. Palmar Sweating as an Index of Autonomic Activity.

Conferences, Meetings, and Symposia

Scores of papers relevant to psychopharmacology have been presented at meetings of scientific societies held during the past few months, and several conferences and symposia have been devoted entirely to psychopharmacological topics. Notes on a few of the meetings and lists of authors and titles of papers of most direct interest to psychopharmacology are presented in the following pages as a general service for readers who did not attend the meetings. The papers cited were selected from program materials available at the meetings Authors' addresses are given, and information on published abstracts of the papers or plans to publish the proceedings of conferences and symposia has been included where possible.

XIV International Congress of Applied Psychology

On August 13-19, 1961, the XIV International Congress of Applied Psychology convened in Copenhagen, Denmark. One of the symposia was devoted to psychopharmacology. The papers presented at the symposium were:

Structure de la personnalité et appreciations psychologiques produites par les drogues psychotropes. Pierre Pichot (France).

Measuring drug-induced changes in emotions and personality. Hannah Steinberg (UK).

Psycho-physiological methods for the study of drug action in man. Ingmar Duremann (Sweden).

Methodologische ueberlegungen zum nachweis psychopharmakologisch induzierter veränderungen der psychomotorik, der psychosensorik, der leistung und der intelligenz. Manfred Pflanz (Germany).

Methods for studying mood changes produced by drugs. Vincent Nowlis (USA).

Other reports, delivered during the course of the Congress and relevant to psychopharmacology, were:

The effects of chlorpromazine on stimulus generalization in psychiatric patients. C. B. Blakemore (Department of Clinical Research, Banstead Hospital, Sutton, Surrey, Great Britain).

Uber die abhängigkeit der wirkung psychotroper substanzen von psychischen faktoren. W. Janke (Marburg/Lahn, Deutschland).

The relationship of the placebo response to the patient's attitude toward physicians and treatment. J. B. Knowles (Medical Research Council's Clinical Psychiatry Research Unit, Chichester, G. B.), and C. J. Lucas (Student Health Centre, University College, London, G. B.).

Analysis of perception of hand position with and without vision, using a drug. D. Legge (Psychopharmacology Unit, Department of Psychology, University College, London).

Experimental analysis of S-R compatibility by means of drugs. A. Summerfield (Psychopharmacology Unit, Department of Psychology, University College, London).

Effect of meprobamate and dextroamphetamine sulphate on the reaction times of normal non-hospitalized subjects to neutral and taboo words. Morgan Upton (Laboratory of Psychopharmacology, Rutgers University, New Brunswick, New Jersey, USA).

The treatment of affective depressions in young women. J. R. Wittenborn (Rutgers University, New Brunswick, New Jersey, USA).

Deutsche Arbeitsgemeinschafte für Neuropsychopharmakologie

On September 1-3, 1961, in Nuremburg, Germany, the Deutsche Arbeitsgemeinschafte für Neuropsychopharmakologie sponsored a symposium on the "Role of Altered Brain Function." This symposium is one of several that have taken place at local meetings of the Collegium Internationale Neuropsychopharmacologium. In a forthcoming issue of *Medicina Experimentale*, the papers presented at the symposium will be published. Inquiries concerning publication should be addressed to the editor of the journal: S. Karger, Arnold-Böcklin-Strasse 25, Basel, Switzerland.

The 41 papers at the symposium covered topics on chemical evaluation of drugs, methodology, drug research with normal subjects and with animals, EEG studies, and the metabolism of drugs. The authors and titles (in the original language and in English) of these papers were as follows:

Heim, F. (Erlangen) Der einfluss einiger pharmaka und gifte auf metaboliten des energiestoffwechsels des gehirns (The influence of several drugs and poisons on metabolites of the brain metabolism).

Sachs, W. and Quadbeck, G. (Homburg, Saar) Psychopharmaka in daueranwendung beim versuchstier (The continuous application of psychopharmaca in animal experiments).

Liebaldt, G. (Tübingen) Bilden antithrombotika eine kontraindikation fur psychopharmaka? (Are psychopharmaca contraindicated in treatment with antithrombotics?).

Werner, G. and Seiler, N. (Marburg, Lahn) Uber die wirkung von MAO-hemmern und anderen psychotropen substanzen auf den oxydativen abbau von methylamin bei einigen säugetieren (The effect of MAO inhibitors and other psychotropic substances on the oxidative breakdown of methylamine in mammals).

Gierhake, R. and Heinze, H. (Giessen) Histochemische untersuchungen mit psychopharmaka (Histochemical investigations with psychopharmaca).

Kraft, H. G. and Hotovy, R. (Darmstadt) Beeinflussung der arbeits- und lernleistung von ratten und tauben durch 2-äthylamino-3-phenyl-norcamphan (The influence of 2-ethylamino-3-phenylnorcamphane on the working and learning ability of rats and pigeons).

Hotovy, R., and Müller-Calgan, H. (Darmstadt) Nachweis der psychomotorischen leistungssteigerung von 2-äthylamino-3-phenyl-norcamphan beim menschen und affen (Demonstration of increased psychomotoric performance in human beings and monkeys caused by 2-ethylamino-3-phenylnorcamphane).

Duker, H. (Marburg, Lahn) Die reaktive leistungssteigerung als störender faktor bei der wirkungsprüfung von schlafmitteln (The reactive increase of working performance as a disturbing factor in testing the effectiveness of hypnotics).

Janke, W. (Marburg, Lahn) Uber die verwendbarkeit objektiv auswertbarer fragebogen bei der erfassung von arzneimittelwirkungen (The usefulness of questionnaires which can objectively be evaluated in testing of drugs).

Balnus, E. (Bonn) Zur objektivierung psychotroper medikamente (On objectification of psychotropic drugs).

Mall, G. and Distler, H. (Landeck) Experimental-psychologische methoden zur erfassung auch schwächerer psychopharmakologischer reaktionen (Methods based on psychological experiments to also evaluate less remarkable [weaker] psychopharmacological reactions).

Mayer, K. (Tübingen) Thioridazinmedikation und fahrtüchtigkeit (Thioridazine medication and driving ability).

Ziolko, H. U. (Berlin) Untersuchungen zum pharmakopsychologischen effekt von centrophenoxin [ANP 235, Helfenberg] (Investigations on psychopharmacological effects with centrophenoxin [ANP 235, Helfenberg]).

Lienert, G. A. (Hamburg) Uber die regression psychischer funktionen als folge pharmakalogischer beeinträchtigung (Regression of psychological functions as a consequence of pharmacological intervention under the influence of LSD).

Holfeld, H. (Göttingen) Psychophysische korrelationen unter der einwirkung von psycholytika [LSD, Psilocibin u. ähnl. (Psychophysical correlations caused by psycholytics [LSD, Psilocybin and similar drugs).

Flügel, F. and Bente, D. (Erlangen) Klinische and elektroencephalographische erfahrungen mit einer neuen zentralwirksamen anticholinergischen droge [Bayer WH 4849] (Clinical and encephalographical experiences with a new centrally active anticholinergic and antiparkinsonistic pharmacological drug [Bayer WH 4849]).

Leuner, H. (Göttingen) Zur ursache von bewusstseinsstörungen in der experimentellen psychose (On the etiology of disturbances of consciousness in experimental psychosis).

v.Ditfurth (Mannheim) Psychomotorisches verhalten und paranoides erleben ein beitrag zur natur des neuroplegischen zustandes (Psychomotoric behavior and paranoid experiences: A contribution to the nature of neuroplegic states).

Hartmann, K. and Hippius, H. (Berlin) Anderungen der psychischen dynamik bei psychosen während der pharmakotherapie (Changes of psychologic dynamics in psychoses during pharmacotherapy).

Degkwitz, R. (Frankfurt) Langfristige selbstversuche mit Tofranil, ein beitrag zur wirkungsweise des Tofranil (Long-lasting experiments with imipramine (Tofranil), a contribution to the way of acting of Tofranil).

Polvan, N. (Istanbul) A neuropsychiatric revision on account of the results obtained with phenothiazine in mental diseases.

Petrilowitsch, N. (Mainz) Uber den indikationsbereich des thioridazin bei der therapie depresiver zustandsbilder (The field of indication for thioridazine in treatment of depressive states).

Bister, W. (Freiburg) Klinische beobachtungen bei der psychosenbehandlung mit haloperidol (Clinical observations in the treatment of psychoses with haloperidol).

Stockhausen, F. G. (Bonn) Weitere klinische erfahrungen mit benzochinolizinderivaten (Further clinical experiences with benzoquinolizine derivatives).

Hirschmann, J. (Tübingen) Phenothiazin in der neurologie (Phenothiazine in neurology).

Denber, H. C. B., Rajotte, P. (New York), and Bente, D. (Erlangen) Wirkungsunterschiede von butyrylperazin bei einer amerikansichen und deutschen patientiengruppe (Differences in the effectiveness of butyrylperazine in an American and German group of patients).

Pfeiffer, W. and Bente, D. (Erlangen) Zur behandlungsdauer bei der medikamentösen therapie endogener depressionen (The duration of treatment with drug therapy in endogen depression).

Harrer, G. (Salzburg) Imipramin und wasserhaushalt (Imipramine and water metabolism).

Schmitt, W. (Homburg, Saar) Uber die abhängigkeit encymatischer aktivitaten von psychopathologischen syndromen und pharmakotherapeutischen massnahmen bei "endogenen" psychosen (Enzyme activities in psychopathological syndromes and pharmacotherapy in endogen psychoses).

Lechner, H., Mayr, F., Geyer, H., and Rodler, H. (Graz) Gehirnzirkulation und psychopharmaca (Blood circulation and psychopharmaca).

Eicke, W. J. (Marburg, Lahn) Beobachtungen an pat. mit therapierefraktären schizophrenen phychosen (Observations on patients with schizophrenia, resistant to therapy).

Flegel, H. (Düsseldorf) Verhaltenswandel während klinisch-psychiatrischer langzeitmedikation, kritisch verglichen mit dem individuellen vorverlauf (Change and behavior during long-term drug treatment of psychiatric patients critically compared with the past history).

Brousolle, P., Thiebaut, E., and Paul, E. (Bron, Lyon) Reflexions sur les longues cures (Thoughts on long-term treatments).

Neumann, H. (Emmendingen) Bemerkungen eines krankenhauspsychiaters zur dauermedikation schizophrener kranker (Remarks on long-term drug treatment of schizophrenic patients given by a hospital psychiatrist).

Fink, M. (Glen Oaks) EEG frequency spectra and psychotropic drug activity. I: Methodologic considerations.

Finckh, R. and Kugler, J. (Munich) Quantitative bestimmung der cerebralen elektrogenese unter dem einfluss von schlafmitteln und thymoleptica (Quantitative determinations of cerebral electrogenesis under the influence of hypnotics and thymoleptics).

Itil, T. (Erlangen) Elektroencephalographische veränderungen bei kombinierter behandlung mit psychotropen drogen und ihre klinische bedeutung (Electroencephalographic changes in combined treatment with psychotropic drugs and their clinical significance).

Ulett, G., Heusler, A., Word, T., Word, V. I., and Quick, R. (St. Louis) The Influence of chlordiazepoxide on drug-altered EEG patterns.

Helmchen, H. and Künkel, H. (Berlin) Pneumencephalographische und elektroencephalographische untersuchungen bei phenothiazin-behandelten (Pneumencephalographic and electroencephalographic examinations in patients treated with phenothiazine).

Helmchen, H. and Künkel, H. (Berlin) Pneumencephalographische und elektroencephalographische untersuchungen bei phenothiazin-behandelten (Pneumencephalographic and electroencephalographic examinations in patients treated with phenothiazine—second report).

Merlis, Sidney (New York) Clinical and electroencephalographic correlates of GABA inhibition in man.

American Psychological Association

The American Psychological Association met in New York City on August 29 to September 6, 1961. Abstracts of the papers appeared in the July 1961 *American Psychologist*, which may be obtained for $1.00 from the APA, 1333 16th Street, N.W., Washington, D. C.

Below is a list of papers on psychopharmacological research which were presented at the meeting. Following the list is a tabulated summary of these papers. The table is, of course, much compressed. In the listing of results, it conveys only the most simple and obvious findings, and omits many details of discussion and of interpretation. Not listed in the table are the papers emphasizing methodology, which were given by Grayson, who discussed traps in psychopharmacological research; Kanter, who described a new self-rating scale for use in studies of depression, and Polidora, who presented his new sequential response test and showed how this measure of complex behavior is useful for studying effects of drugs.

In addition to these individual research reports, there was a symposium entitled the "Alteration of Perceptual Consciousness by Drugs," in which two psychologists, Frank X. Barron and Timothy Leary, and two authors, William Burroughs and Gerald Heard, participated. They addressed themselves to a variety of theoretical issues that ranged from the methodological to the philosophical. Barron described some of the perceptual aberrations reported by subjects who had taken psilocybin. He pointed out that in many cases the predominant features of the experience could have been predicted from a knowledge of the strong perceptual habits—both occupational and cultural—which the subject brought to the situation.

Leary reported that he was skeptical about the validity of the questionnaire data which he had collected in psilocybin experiments. He acknowledged the presence of a volunteer bias and suggested that his results could be attributed to the operation of three main factors: (1) the situation in which the drugs were administered; (2) the suggestibility of the subjects; and (3) the expectations with which the subjects entered the experiment.

Burroughs presented a conceptual scheme for classifying drugs that alter perceptual consciousness. He argued for a distinction between drugs that distort or interfere with the normal perceptual process and those that enhance, intensify, or broaden perceptual experience.

Heard gave a vivid account of his subjective experiences under LSD, and then discussed the philosophical implications of drugs that alter perceptual consciousness. He reported that the general effects of LSD are fairly stable but the specific effects vary as a function of environmental circumstances. All the participants challenged the notion that the psychotomimetics produce lasting changes in personality.

AUTHORS AND TITLES OF PAPERS

Ansfield, Paul J. and Johnson, John I., Jr. (Purdue University and Marquette University) Differential effects of LSD-25 in blind and normal rats.

Barry, Herbert, III, Wagner, Allan R. and Miller, Neal E. (Yale University) Effects of alcohol and sodium amytal on learning and on extinction.

Doyle, Gerald A. (Western Reserve University) Effects of dl-, d-, and l-amphetamine and hunger on exploratory behavior and latent learning in rats.

Feldman, Robert S. (University of Massachusetts) The prevention of behavior rigidity with chlordiazepoxide.

Ferguson, Donald C. and Fisher, Alan E. (University of West Virginia and University of Pittsburgh) Precision timing behavior in *Cebus apella* as a function of intraperitoneal injections of normal, stressed normal, and catatonic schizophrenic serum and plasma.

Fisher, Alan E. and Davis, Philip. (University of Pittsburg) Neuprobiochemical mediation of eating and drinking behavior.

Grayson, Harry M. (Neuropsychiatric Hospital, Los Angeles, California) Traps in psychopharmacological research.

Grossman, Sebastian P. (Yale University) Effects of adrenergic and cholinergic blocking agents on eating and drinking elicited normally or by chemostimulation of the brain.

Hargreaves, William A. and Starkweather, John A. (Langley Porter Neuropsychiatric Institute and University of California School of Medicine) Drug effects on vocal behavior.

Kanter, Victor B. (London, England) A self-assessment inventory used to measure depression and recovery in outpatients treated with imipramine (Tofranil).

Mize, Donna and Isaac, Walter (Emory University) The effects of sodium pentobarbital and d-amphetamine on the latency of the simple avoidance response in the rat.

Nash, Harvey (Chicago, Illinois) Psychological effects of amphetamines and barbiturates.

Overton, Donald (McGill University) Discriminative behavior based on the presence or absence of drug effects.

Polidora, V. J. (University of Wisconsin Primate Laboratory) An automated sequential response test and its application to the study of drug effects on complex behavior.

Ray, Oakley S. and Marrazzi, Amedeo S. (V.A. Research Laboratories in Neuropsychiatry, Pittsburgh, Pennsylvania) Psychotogen effects on approach and avoidance behavior in rats.

Smith, G. M. and Beecher, H. K. (Harvard Medical School) Objective and subjective effects of morphine and heroin.

Stern, Muriel H., Routtenberg, A. S. and Steinberg, June (McGill University and Jewish General Hospital, Montreal, Canada) Effects of the blood of schizophrenics on rat behavior.

Waller, Marcus B. and Waller, Patricia F. (Roscoe B. Jackson Memorial Laboratory) Effects of chlorpromazine on a multiple schedule having both appetitive and aversion components.

Weaver, Lelon A., Jr. (University of Vermont College of Medicine) The relation of drug induced extrapyramidal motor system dysfunction to performance on a battery of psychomotor tests.

Weiner, Harold (St. Elizabeths Hospital, Washington, D. C.) The effects of "unwanted" signals and dextro-amphetamine sulfate on observer responses.

Authors	"Drugs"	Subjects	Behavior	Results
Ansfield and Johnson	LSD	Blind and normal rats	Learning in a Hebb-Williams maze	At high doses LSD reduced learned behavior more in blind than in normal rats
Barry, Wagner and Miller	ethanol and amobarbital	Rats	Speed of response during learning and extinction trials in a straight alley	Drugs, compared with placebo, increased performance during early acquisition and extinction but final level of performance was the same
Doyle	d-, l-, and dl-amphetamine	Hungry and satiated rats	Exploration and latent learning	Drug increased exploratory and subsequent learning, with d- and l- forms more effective than dl-
Feldman	Chlordiazepoxide	Rats	"Response fixation" developed in an insoluble, two-choice problem	Drug lowered response fixations
Ferguson and Fisher	Blood serum and plasma from catatonic schizophrenics and from normals and stressed normals	Cebus monkey	Precision timing behavior	Substances from schizophrenics and stressed normals increased time to complete task
Fisher and Davis	Adrenergic and cholinergic substances	Rats with implanted cannulae	Eating and drinking	Cholinergic substances increased intake of water but not food, or increased both, depending on brain placement; adrenergic substances had no consistent effect
Grossman	Atropine and ethoxybutamoxane	Hungry and thirsty rats, under chemostimulation of brain	Eating and drinking	Atropine eliminated cholingeric drinking effect, and ethoxybutamoxane almost completely eliminated the adrenergic eating effect
Hargreaves and Starkweather	Sodium pentobarbital	Undergraduate women	Oral reading and reaction time	Reaction time increased, verbal fluency decreased, intensity of voice was related to dosage, but voice frequency was not
Mize and Isaac	Sodium pentobarbital and d-amphetamine	Rats	Latency of escape in a shuttle box	Latency lengthened by pentobarbital and shortened by amphetamine
Nash	d-amphetamine	Volunteers	"Intellectual functioning"	Dose containing amphetamines had "beneficial" objective and subjective effects, but sedative dose of barbiturate had no significant effects
Overton	Sodium pentobarbital and Flaxedil	Rats	Ability to make discriminations with and without effects of drugs, to escape shock in a T maze	Animals easily learned to discriminate under influence of sodium pentobarbital, but not under Flaxedil, with pentobarbital also showing transfer effects to other drug states
Ray and Marrazzi	LSD and mescaline	Rats	Lever pressing to avoid shock or to obtain food, with auditory signal as discriminatory stimulus	Both drugs reduced response to milk but not to avoid shock
Smith and Beecher	Heroin and morphine	Healthy non-addicted college men	Cognitive and subjective effects, measured by tests and a questionnaire	Heroin impaired all test performances, and produced intense and earlier unpleasant side effects than morphine
Stern, Routtenberg and Steinberg	Blood, formed elements, and plasma from schizophrenics and normals	Rats	Visual discrimination and competition for food	Whole blood of both groups had deleterious effect, with effects of schizophrenic blood significantly worse, schizophrenic, but not normal, plasma lowered performance, and formed elements had no effect
Waller and Waller	Chlorpromazine	Rats	Lever pressing on multiple schedule, with both avoidance and approach components	Drug showed no differential results on the two components
Weaver	Tranquilizer	Chronic schizophrenics with drug-induced extrapyramidal dysfunction	Psychomotor	Tests reflected extrapyramidal dysfunction, especially of precision and fine coordination
Weiner	d-amphetamine	Male undergraduates	Vigilance in monitoring a scope, with "wanted" and "unwanted" signals	"Unwanted" signals increased response, and placebo and amphetamine increased it even more

*American College of Neuropsychopharmacology**

The first organizational meeting of the American College of Neuropsychopharmacology (A.C.N.P.) was held in Washington, D. C., October 7-8, 1961, under the chairmanship of Dr. Theodore Rothman. Dr. Jonathan O. Cole spoke briefly on the need for an American College of Neuropsychopharmacology, and Dr. Paul H. Hoch discussed the proposals and objectives for the college.

The 90 participating members recommended that a multi-disciplinary group composed of 122 persons be accorded temporary Charter Fellowship. However, a credentials committee will study all temporary fellowships before permanent Charter Fellowship in the A.C.N.P. will be offered. Furthermore, no new fellowships will be considered until some future date.

During the course of the meeting, Dr. Joel Elkes moderated a symposium on the contributions of the basic sciences to clinical neuropsychopharmacology; Dr. Fritz Freyhan moderated a symposium on the contributions of the clinician to the basic scientist in clinical neuropsychopharmacology; and Dr. Jonathan O. Cole spoke on the "Current Program of the Psychopharmacology Service Center, National Institute of Mental Health."

The participating members, representing 22 States and 2 Canadian provinces, approved a constitution and by-laws for the American College. All disciplines immediately concerned with neuropsychopharmacology, including pharmacology, psychiatry, psychology, neurophysiology, and biochemistry were represented at the meeting.

The officers elected by the A.C.N.P. at this meeting are: President, Dr. Joel Elkes; President-Elect, Dr. Paul H. Hoch; Vice-President, Dr. Klaus R. Unna; Secretary-Treasurer, Dr. Theodore Rothman; and Assistant Secretary-Treasurer, Dr. Milton Greenblatt. The council members elected for 1, 2 and 3 year terms are: Dr. Frank J. Ayd, Jr., Dr. Bernard B. Brodie, Dr. Heinz E. Lehmann, Dr. Joseph Zubin, Dr. James E. P. Toman, and Dr. Jonathan O. Cole.

The first meeting of the Executive Council of the A.C.N.P. will be held at the Barbizon-Plaza Hotel in New York City, December 9, 1961.

First International Pharmacological Meeting

The First International Pharmacological Meeting was held in Stockholm, Sweden, August 22-25, 1961, under the auspices of the section on pharmacology of the International Union of Physiological Sciences.

The central theme of the meeting was the *mode of action of drugs*. The program of the meeting was organized around eight major symposia: drugs and membranes, pharmacological control of release of hormones including antidiabetic drugs, modern concepts in the relationship between structure and biological activity, methods for the study of pharmacological effects at cellular and subcellular levels, new aspects of cardiac glycosides, pharmacological analysis of central nervous action, metabolic factors controlling duration of drug action, and effects of drugs on synthesis and mobilization of lipids. Each of the symposia will be published separately in collaboration with the Pergamon Press, Ltd., Oxford, England. Inquiries regarding publication should be addressed to Pergamon.

Of the nearly 600 separate reports presented at the meeting, more than 40 of them pertained to psychopharmacology. The authors and titles of these papers are given below. The entire program of the meeting was published in *Biochemical Pharmacology* 8, August 1961.

CLINICAL EXPERIMENTS, REFLEXES, CONDITIONING

Controlled environment: A method of measuring the drug-response behavior in humans. J. C. Pollard (Mental Health Research Inst., Ann Arbor, Mich., U.S.A.) and L. Uhr (U.S.A.)

Central autonomic reactivity in humans: Its alteration by motor activity, adrenaline, and CNS depressing and tranquillizing agents, e.g., amobarbital sodium, Nevental, meprobamate, carisoprodol. K. Rickels (Hospital of the Univ. of Pennsylvania, Philadelphia, Penna.), J. H. Ewing and F. Fochler (U.S.A.).

The action of Miltown on rats trained to concurrent variable-ratio avoidance schedules. L. Molinengo (Dept. of Pharmacology, Univ. of Turin, Turin, Italy).

Individual variations of pharmacological central reactions. G. Tonini (Institute of Pharmacology, Bologna Univ., Bologna, Italy) and M. Babbini (Italy).

Electroencephalographical discrimination between sedato-hypnotic and major tranquillizing effects in rats with non-extinguishable conditioned reflex. K. Kelemen (Inst. of Pharmacology, Univ. of Budapest, Budapest, Hungary), K. Magyar and J. Knoll (Hungary).

A new technique for studying the inhibitory effects of drugs on discriminatory avoidance-escape habits in rats. C.J.E.J. Niemegeers (Research Laboratory Dr. C. Janssen, Beerse, Belgium).

* Prepared by Dr. Theodore Rothman, American College of Neuropsychopharmacology.

The action of some CNS depressants and stimulants on the "carried" extinction in rats. E. Beccari (Dept. of Pharmacology, Univ. of Turin, Turin, Italy), and L. Molinengo (Italy).

Assessment of pharmacological actions upon the brain, based upon experimental studies of habituation. E. M. Glaser (Evans Medical Laboratories, Liverpool, Great Britain).

Studies on the functional state of brain in the general action of procaine. G. I. Zobcallo (Pavlov Inst. of Physiology, Academy of Sciences U.S.S.R., Koltushi, U.S.S.R.) and T. M. Kucherenko (U.S.S.R.).

The effects of vitamin B_{12}, B_6 and procaine on the conditioned escape response. R. Kiesewetter (VEB Jenapharm, Jena/Thuringen, East Germany), R. Itterheim and J. Gebhard (Germany).

Pharmacological observations on the responses to afferent stimulation of the vagus nerve. J. A. Izquierdo (Fac. de Farmacio y Bioquímica, Universidad de Buenos Aires, Argentina), A. J. Kaumann, J. D. Coussio and I. Izquierdo (Argentina).

Effects of drugs on timing behavior. G. Maffi (Dept. of Pharmacology, Lepetit S.p.A., Res. Labs., Milan, Italy) and D. Costantini (Italy).

PHENOTHIAZINE DERIVATIVES

Mode of action of chlorpromazine. P. S. Guth (Dept. of Pharmacology, Tulane Univ., School of Medicine, New Orleans, La., U.S.A.) and M. A. Spirtes (U.S.A.).

Effect of largactil on central nystagmus. F. Bergmann (Dept. of Pharmacology, Medical School, Jerusalem, Israel), J. Lachmann, M. Chaimovitz and J. Gutmann (Israel).

The neuroplegic effect of Frenolon, a new phenothiazine compound. J. Borsy (Research Inst. for the Pharmaceutical Industry, Budapest, Hungary), and L. Toldy (Hungary).

On the relation between the ataractic and some other types of central action of some phenothiazine derivatives. B. I. Liubimov (Inst. of Pharmacology and Chemotherapy, Moscow, U.S.S.R.) and K. S. Raevsky (U.S.S.R.).

On the influence of the neuroplegic drugs on chemoreception. U. I. Vikhliaev (Inst. of Pharmacology and Chemotherapy, Moscow, U.S.S.R.).

Photochemical conversion of phenothiazines. G. Urban (Inst. f. Pharmakologie und Pharmazie der Vet.-Med. Fakultat der Humboldt-Universitat, Berlin, East Germany).

TREMOR-INDUCING DRUGS, ANTICONVULSANTS, GABA

Extraction of a seizure substance (K substance) from the dog's brain. T. Hayashi (Dept. of Physiology, School of Medicine, Tokyo, Japan) and K. Nagai (Japan).

The physiological action of K substance extracted from dog's brain. T. Hayashi (Dept. of Physiology, School of Medicine, Tokyo, Japan) and K. Nagai (Japan).

On the activity of Y-amino-butyric-acid derivatives on central nervous system excitability. A. Bertelli (Inst. of Pharmacology, University of Milan, Milan, Italy) and G. Gavazzi (Italy).

Tranquilizers and antidepressants: A pharmacological comparison. F. Herr (Ayerst Research Labs., Montreal, Canada), J. Stewart and M. P. Charest (Canada).

Differentiation of central depressants by means of the veratramine excitation. W. Schoetensack (Research Dept., Fa. Byk-Gulden, Konstanz a. B., West Germany) and G. Hallmann (Germany).

Three types of artificially induced tremors and the effect of some antiparkinsonian agents upon them. I. L. Bonta (N. V. Organon, Pharmacol. Dept., Oss, Netherlands) and H. M. Greven (Netherlands).

A contribution to the pharmacology of tremorine-induced tremor. P. Stern (Inst. for Pharmacology, Medical Faculty, Sarajevo, Yugoslavia) and J. Gasparovic (Yugoslavia).

EEG TECHNIQUES

Contribution to the study of cerebral maturation in animals. C. Radouco-Thomas (Faculty of Medicine, University of Geneva, Geneva, Switzerland), F. Martin, S. Radouco-Thomas, G. Nosal and J. M. Chaumontet (Switzerland).

Relationship between reserpine-induced facilitation of evoked potentials in the limbic system and change in brain serotonin level. A. M. Revzin (National Institutes of Health, Bethesda, Md., U.S.A.), S. Spector and E. Costa (U.S.A.).

Pharmacology of hippocampus pyramidal cells. F. Brücke (Pharmakol. Inst. der Universitat, Vienna, Austria) and C. Stumpf (Austria).

Stereotaxic neuro-psycho-pharmacology-electrographic analysis and neurovegetative action of the N-N'-diethylamide of the 2-methoxy-4-allylphenoxyacetic acid (G.29505) during stereotaxic functional investigations in man. J. Bancaud, M. Rebufat-Deschamps (Université de Paris, Cliniques des Maladies Mentales et de l'Encephale, Paris, France), J. Thuillier and P. Deligné (France).

An analysis of tranquilizers in chronically electrodes-implanted cat. R. Kido (Shionogi Research Laboratory, Amagasaki, Japan) and K. Yamamoto (Japan).

The effect of mescaline on the optic evoked potential. J. R. Smythies (Maudsley Hospital, London, Great Britain), W. P. Koella and C. K. Levy (U.S.A.).

TRYPTAMINE DERIVATIVES, RESERPINE

Effect of reserpine and the metabolism of serotonin in tryptophan deficient rats. E. M. Gal (Dept. of Pharmacology, University of California Medical School, Los Angeles, Calif., U.S.A.), P. A. Drewes and C. A. Barraclough (U.S.A.).

Amino-acid and carbohydrate metabolism of brain after reserpine. D. Palm (Pharmakol. Inst. der Universitat Frankfurt am Main, Frankfurt am Main, West Germany), H. Balzer and P. Holtz (Germany).

Reserpine: modification of its tranquilizer effect and analysis of its central mode of action. J. Knoll (Pharmacological Inst., Univ. of Budapest, Budapest, Hungary) and B. Knoll (Hungary).

CHLORDIAZEPOXIDE, IMIPRAMINE, NEW DRUGS

Pharmacology of chlordiazepoxide (Librium) and analogues. L. O. Randall (Hoffman-La Roche Inc., Nutley, N.J., U.S.A.) and B. Kappell (U.S.A.).

Effects of chlordiazepoxide (Librium) and other psychopharmacological agents on "fixated" behavior in rats. W. T. Liberson (V.A. Hospital, Hines, Ill., U.S.A.), A. Kafka and E. Schwartz (U.S.A.).

On mechanism of the antidepressant action of imipramine. F. Sulser and B. B. Brodie (National Heart Institute, Bethesda, Md., U.S.A.).

Influence on a new group of tranquilizers, derivatives of dibenzosuberene, on the central nervous system. Z. Votava (Res. Inst. for Pharmacy and Biochemistry, Prague, Czechoslovakia), J. Metysová and M. Sousková (Czechoslovakia).

Pharmacological action of a new antidepressant drug, propazepine, in comparison with imipramine. J. Metysová (Res. Inst. for Pharmacy and Biochem., Prague, Czechoslovakia) and Z. Votava (Czechoslovakia).

Mode of action of centrophen-oxin and derivatives in fish: behavior and chromatophores. H. Nakajima (Hôpital Ste. Anne, Paris, France), J. R. L. L'Huillier, L. Bajinski and J. Thuillier (France).

Pharmacological analysis of concomitant narcosis and respiratory stimulation induced by a single drug. J. Thuillier (Unite de Recherches de Psycho-Pharmacologie de l'Institut National d'Hygiène, Paris, France).

MAO, CATECHOLAMINES

Non-hydrazide monoamine oxidase inhibitors and their effects on central amines and motor behavior. G. M. Everett (Abbott Laboratories, North Chicago, Ill., U.S.A.) and R. G. Wiegand (U.S.A.).

Pharmacology and biochemistry of x-methyl-dopa in man and experimental animals. A. Sjoerdsma (National Heart Inst., Bethesda, Md., U.S.A.) and S. Udenfriend (U.S.A.).

The American Society for Pharmacology and Experimental Therapeutics

The American Society for Pharmacology and Experimental Therapeutics met in Rochester, New York, on August 29 to September 1, 1961. Abstracts of the papers were published in the Fall issue of *The Pharmacologist* 3, 1961, copies of which may be obtained for $1.00 by writing to Dr. H. George Mandel, George Washington University, School of Medicine, 1335 H Street, N.W., Washington 5, D.C.

Below is a brief list of those papers that were given, and that are relevant to psychopharmacology. They are listed by title of session in which they were given.

CELLULAR AND BIOCHEMICAL PHARMACOLOGY

Metabolism of ethanol. M. Samir Amer and Martin P. Schulman (University of Illinois).

The effect of chlorpromazine on release of neurohumors in vitro. Paul S. Guth and Andrew G. Finlay (Tulane University).

Competitive inhibition of dihydroxyphenylalanine (DOPA) decarboxylase in vitro. E. C. Griesemer, R. M. Fleming, W. J. Hartman, and W. G. Clark (V.A. Hospital, Sepulveda, and U.C.L.A. Medical Center).

Effect of 5-hydroxytryptamine (5-HT) on glycolysis in lamellibranch gill. Kenneth E. Moore and Robert E. Gosselin (Dartmouth Medical School).

DRUG METABOLISM

Mechanism of meprobamate tolerance in the rat. Barrie M. Phillips, Tom S. Miya, and George K. W. Yim (Purdue University).

The fate and excretion of d-amphetamine in man. Rex Pinson, Jr., Josephine Chiaini, and Doris Baumgartner (Chas. Pfizer and Co., Inc.).

Metabolism of a GABA transaminase inhibitor, aminooxyacetic acid (AOAA). G. R. Zins (The Upjohn Company).

ENDOCRINOLOGY

Inhibition of the reserpine-induced ACTH hypersecretion with a monoamine oxidase inhibitor (N-methyl-N-benzyl-propynylamine). R. Martel, and R. P. Maickel (National Institutes of Health).

NERVE AND MUSCLE

The action of phenothiazines on the end plate potentials of frog muscle. W. Schlosser, and C. P. Kraatz (Jefferson Medical College).

NEUROPHARMACOLOGY

Some factors modifying the effects of eserine on cortical evoked potentials. W. D. Winters, J. Siegel, and A. J. Hance (U.C.L.A. Medical Center).

Brain cholinesterase inhibition after dimethyl carbamate of deoxy demethyl lycoramine, physostigmine or neostigmine. M. M. Hein, and R. L. Irwin (National Institute of Neurological Diseases and Blindness).

Synaptic inhibition by serotonin in isolated cerebral cortex. Jose M. Rodriguez, E. Ross Hart, and Amedeo S. Marrazzi (V.A. Research Laboratories in Neuropsychiatry, Pittsburgh).

Central and peripheral effects of amitriptyline and ethyltryptamine. H. Sabelli, J. Levin, L. Seigler, and J. Toman (Chicago Medical School).

Effects of CO_2 on reflex and central control of respiration in cats. R. Rosenstein, and H. L. Borison (University of Utah).

Characterization of rigidity following spinal cord ischemia. Satoshi Murayama, and Cedric M. Smith (University of Illinois).

The effect of CNS stimulants upon pentobarbital depression in dogs. A. Kandel, R. B. Miller, C. A. Leonard, and E. J. Fellows (Smith Kline and French Laboratories).

The analeptic action of adrenergic neurone blocking agents in barbiturate depression. Alexander H. Friedman, and A. S. Kulkarni (University of Wisconsin).

Neuropharmacology of dl-alpha-methyl-p-trifluoromethyl-phenethylamine hydrochloride (SK&F No. 7410-A). C. A. Leonard,

T. Fujita, D. H. Tedeschi, and E. J. Fellows (Smith Kline and French Laboratories).

Differential effects of Dilantin on various components of the pyramidal response. Baruch Blum (Montreal Neurological Institute).

Autonomic agents that cause death after supramaximal electroshock in mice. C. D. Hendley, T. M. Mikiten, and S. Irwin (Schering Corp.).

Effect of electrical stimulation on the neural synthesis of noradrenaline from L-tyrosine-C^{14}. W. G. Clark, F. J. Kotichas, H. F. Schott, D. T. Masuoka, and W. Drell (V.A. Hospital, Sepulveda, and U.C.L.A. Medical Center).

Sensitivity of the nictitating membrane after denervation and after various drugs. U. Trendelenburg, and N. Weiner (Harvard Medical School).

Modification of afferent interaction patterns by chlorpromazine, meprobamate, morphine and pentobarbital. C. L. Mitchell, S. D. Nelson, and K. F. Killam (Stanford University).

A technique for the classification and comparison of drug effects on the cat with chronically implanted electrodes. Zola P. Horovitz, and May-I Chow (Squibb Institute for Medical Research).

The pseudaffective response to intra-coronary injection of bradykinin and other algesic agents. Frank Guzman, C. Braun, and R. K. S. Lim (Miles Laboratories, Inc.).

PHARMACOLOGY

Clinical Pharmacology and Psychiatry. Douglas Goldman (Cincinnati, Ohio) (Banquet address).

PSYCHOPHARMACOLOGY

Antagonism of lysergic acid diethylamide (LSD)-induced hyperthermia. John T. Elder, and M. Kent Shellenberger (University of Washington).

Drinking induced by cholinergic stimulation of hypothalamus is a muscarinic, not nicotinic, action. Larry Stein, and Joseph Seifter (Wyeth Laboratories, Inc.).

A treadmill device for studying centrally active compounds in rats. Melvyn I. Gluckman (Wyeth Institute for Medical Research).

LAIII, a new psychotropic drug. R. F. Banziger (Hoffmann-La Roche, Inc.).

Some effects of scopolamine, atropine and amphetamine in three behavioral situations. Peter L. Carlton (The Squibb Institute for Medical Research).

Interaction effects of imipramine and d-amphetamine on non-discriminated avoidance. Albert Weissman (Chas. Pfizer & Co., Inc.).

The effect of phenelzine on the pharmacology and toxicology of various drugs. John Shanaman, Jane Frances Emele, V. Carolyn Scott, W. E. Rowe, M. R. Warren, and C. C. Scott (Warner-Lambert Research Institute).

Comparison of tranquilizers by human bioassay. T. Bodi, P. E. Siegler, J. W. Slap, R. Khorsandian, and J. H. Nodine (Hahnemann Medical College and Hospital).

Biochemical correlates of pharmacologic activity. H. Green, R. W. Erickson, and J. L. Sawyer (Smith Kline & French Laboratories).

Practice as a variable in altering the effects of drugs. Conan Kornetsky, and Joy Dawson (Boston University).

Impairment of cardiac and motor conditional reflexes after encephalopathy produced by anti-dog brain serum. Clara H. Williams, and W. Horsley Gantt (V.A. Hospital, Perry Point, Maryland).

Behavioral and biochemical activity of a new series of benzoquinolizine derivatives. K. F. Finger, A. Weissman, and J. R. Tretter (Chas. Pfizer & Co., Inc.).

Disruption of the conditioned avoidance response of trained rats with 5-methoxy-N:N-dimethyltryptamine. P. K. Gessner (Cleveland Clinic Foundation and the Frank E. Bunts Educational Institute).

The effects of several drugs on gross motor activity of mice exposed to ether. Max A. Heinrich, Jr., and Donal M. Moe (North Dakota State University).

Differential tolerance development to the avoidance and locomotor suppressant actions of perphenazine and chlorpromazine. S. Irwin (Shering Corp.).

Comparison of some pharmacological properties of imipramine, amitriptyline, promazine and their desmethyl derivatives. E. B. Sigg, L. Gyermek, and L. Soffer (Geigy Research Laboratories).

Potentiation of methylphenidate by imipramine, amitriptyline and their desmethyl analogues. R. T. Hill, I. Koosis, M. W. Minor, and E. B. Sigg (Geigy Research Laboratories).

A systematic evaluation of the effects of monourethanes, diurethanes and barbiturates on experimentally induced conflict. Irving Geller, and Joseph Seifter (Wyeth Laboratories, Inc.).

White rats as subjects for anorexic tests. George A. Emerson (University of Texas Medical Branch, Galveston).

A quantitative procedure for assessing appetite depressant activity of drugs. Adolph P. Roszkowski, and Nancy M. Kelley (McNeil Laboratories, Inc.).

RELEASE OF CATECHOLAMINES

Studies on the storage and release of the catecholamines of the adrenal medulla. W. R. Burack (Harvard Medical School, Boston).

The uptake, release and metabolism of H^3-norepinephrine and and H^3-epinephrine. J. Axelrod (National Institutes of Health, Bethesda).

Chronotropic action of reserpine and guanethidine in the heart-lung preparation of the dog. O. Krayer (Harvard Medical School, Boston).

The release of catecholamines from the isolated heart by mephentermine. C. R. Swaine (Woman's Medical College, Philadelphia 29).

Release of catecholamines from nerve endings as a mechanism of drug action. M. Nickerson (University of Manitoba, Winnipeg 3).

TOXICOLOGY

Effect of thioridazine on canine hepatic blood flow. Gabriel L. Plaa, Eileen T. Eckhardt, and Thomas D. Darby (Tulane University).

Effect of phenothiazines on hepatic blood flow in the mouse. Eileen T. Eckhardt, and Gabriel L. Plaa (Tulane University).

Third World Congress of Psychiatry

Advances in clinical and basic psychopharmacology were actively discussed throughout the week-long Third World Congress of Psychiatry held June 4-10, 1961 at Montreal, Canada. Whereas the previous congress in Zurich, Switzerland in 1959 had devoted its program to "schizophrenia", the Third World Congress ranged broadly over many topics. Over 3,000 psychiatrists and other mental health professionals from all over the world, including the U.S.S.R., participated in the program of what was almost universally agreed to have been a well-planned and exceptionally well-executed meeting.

While papers on psychopharmacology were prominent throughout the program, the high point was Thursday, June 8, when a special panel organized by J. Delay, Paris, France, reviewed the broad scope of clinical problems. Special issues discussed by H. Brill, H. E. Lehmann, P. Deniker, R. Kuhn, M. Greenblatt, and F. A. Freyhan at this panel served as topics for seven sessions which met concurrently that afternoon. The sessions discussed:

1. The impact of modern chemotherapy on hospital organization, psychiatric care, and public health policies.
2. Selection, screening, and testing of new psychiatric drugs.
3. Implications of neurological effects of psychiatric drugs. The problem of toxicity in chronic administration.
4. The modification of symptomatology and evolution of mental diseases produced by chemotherapy. Phenomenology and psychodynamics of the therapeutic process.
5. Combination of chemotherapy with the other methods of psychiatric therapy.
6. Methods of classifying psychiatric drugs.
7. Specific and nonspecific factors in psychopharmacology.

Although relatively few striking new drugs or findings were reported upon, these sessions provided a systematic overview of the rapidly changing areas of clinical drug therapy.

Another special panel held Thursday evening, June 5th, was devoted to "EEG Changes in Human Psychopharmacology". This panel, organized by Max Fink, M.D., brought together North American and European workers who have in recent decades evolved a number of convergent approaches which offer promise of clarifying the effects of drugs on EEG patterns. Members of this panel also participated in special seminars this summer in Germany and Italy, and they report unusual progress.

The papers relevant to psychopharmacology that were given at the Congress are listed below. The titles are taken from the program guide; we have made no attempt to translate titles that did not appear in English. Abstracts of the meeting were printed in 2 parts by the Abstracts Printing Committee, chaired by Dr. W. J. Stauble, and printed by L. J. Dunbar & Co., of Canada. Anyone wishing to receive copies of the abstracts should send a money order in the amount of $2.50 either U. S. or Canadian funds, payable to the Third World Congress of Psychiatry, and should direct their request to Dr. C. A. Roberts, 6875 La Salle Blvd., Verdun, P. Q., Canada.

(*Note:* The names of the speakers who presented the papers are given first; co-authors appear in parentheses; the papers are grouped by title of session in which they were given; only papers concerned with psychopharmacology are listed.)

AFTERCARE

Kris, E. B. New York, New York, USA
Aftercare and rehabilitation of patients returned to the community.

ALCOHOL AND DRUG DEPENDENCY

Lemere, F. Seattle, Washington, USA
The use of drugs on the treatment of alcoholism.

BIOCHEMISTRY

Arnold, O. H. Vienna, Austria (G. Hoffmann)
Results of a biochemical test-method for the diagnosis of schizophrenic phychoses and their hereditary taint.

Bogoch, S. Boston, Mass., USA
Nervous system glycoproteins in mental disorder.

Drwal, T. Lublin, Poland (A. Jasinski)
Chromatographic researches of cerebro-spinal fluid in early schizophrenia treated with chlorpromazine.

Feldstein, A. Shrewsbury, Mass., USA (M. R. Riveria, H. Hoagland, H. Freeman)
The effect of antidepressant drugs on serotonin metabolism.

Freedman, D. X. New Haven, Conn., USA
Studies of LSD-25 and serotonin in the brain.

Grof, S. Prague, Czechoslovakia (M. Vojtechovsky, V. Vitek, S. Frankova)
Clinical and experimental study of central effects of adrenochrome.

Haddad, R. K. Princeton, N. J., USA (A. Rabe)
An anaphylactic test for abnormal antigen (s) in schizophrenic serum.

Heath, R. G. New Orleans, La., USA (B. E. Leach, L. W. Byers)
Taraxein: recent developments in processing and identification.

Huszak, I. Szeged, Hungary (L. Durko)
The metabolism of the indol compounds in schizophrenia.

Kaelbling, R. Columbus, Ohio, USA (J. B. Craig, B. Pasamanick)
Screening of 2,500 psychiatric patients for urinary porphobilinogen.

Martens, S. Stockholm, Sweden (B. Melander)
Experimental and clinical properties of the combination tranylcyprominemylcamate.
Resnick, O. Shrewsbury, Mass., USA (M. R. Rivera, D. Engels, H. Freeman)
Biochemical effects of antidepressant drugs in depressed patients.
Sankar, D. V. S. Jamaica, New York, USA (E. Gold, E. Phipps, D. B. Sankar)
Biochemical studies on schizophrenic children.
Scarpalezos, S. Athens, Greece
Aspects psychiatriques particuliers de la porphyrie.
Schenker, V. Brooklyn, New York, USA (G. Marjerrison, P. Schlachet, N. Freedman, L. D. Hankoff, D. M. Engelhardt).
M.A.O. inhibition and anti-depressive correlates in hospital patients.
Sourkes, T. L. Montreal, Canada (G. F. Murphy, B. Chavez)
Experimental and clinical studies of anti-decarboxylases.
Szara, S. Washington, D. C., USA (L. H. Rockland)
Psychological effects and metabolism of N.N.-diethyl-tryptamine—an hallucinogenic drug.
Wortis, J. Brooklyn, New York, USA (E. Jackim)
Metabolic studies in mongolism.

CHILDREN

Fish, B. New York, New York USA
The influence of maturation and abnormal development on the responses of disturbed children to drugs.
Knobel, M. Buenos Aires, Argentina
Dynamic considerations on psychopharmacology for the hyperkinetic child.

CONCEPTS AND METHODS

Cochrane, C. M. Chapel Hill, N. C., USA (A. J. Prange, D. W. Abse)
Changes in the direction of aggressive drives following reserpine treatment.
Von Ditfurth, H. Mannheim, Germany
Besonderheiten des wirkungsverlaufes antipsychotischer pharmaka.
Lewis, N. D. C. Princeton, N. J., USA (I. Bird, D. Boyle, J. Tobin)
The conduct of psychopharmacological research in an outpatient setting.

DEPRESSION

Angst, J. Zurich, Switzerland
Tofranil: a clinical analysis.
Degkwitz, R. Frankfurt, Germany (Ehrenreich, Schemmann, Schmoll)
The effect of imipramine (Tofranil) in different kinds of depression in comparison with electro-shock treatment—a report on 600 cases.
Flach, F. F. New York, New York, USA (C. Burrell, E. Liang)
Calcium metabolism in depressive reactions.
Kaplan, S. M. Cincinnati, Ohio, USA (R. Stuart, Kravets, W. D. Ross)
The effects of imipramine on the depressive components of medical disorders.
Rothman, T. Los Angeles, Calif., USA (H. Grayson, J. Ferguson)
Autonomic and other changes associated with isocarboxazid (Marplan) and imipramine (Tofranil) in the treatment of the depressive syndromes.
Sainz, A. New York, New York, USA (N. Bigelow, J. Schneider)
Relative antidepressant activity of various chemical groups.

EEG

Marczak, R. Lublin, Poland (A. Majczak)
Experiments on the significance of electroencephalographic changes under the influence of chlorpromazine of patients in early stages of schizophrenia.

FORENSIC

Goldner, R. D. Saginaw, Michigan, USA
Control of minor criminal sexual compulsions with imipramine and amine oxidase regulators.

GENETICS

Hurst, L. A. Johannesburg, S. Africa
Research implications of converging advances in psychiatric genetics and the pharmacology of psychotropic drugs.

NEUROPHYSIOLOGY

Himwich, H. E. Galesburg, Ill., USA (G. G. Brune)
Effects of reserpine and isocarboxazid on behavior of mental patients and serotonin metabolism.
Popov, E. A. USSR
Some mental diseases and their relation to the phenomena of sleep, anaesthesia, and hibernation.

PHYSICAL THERAPIES

Caffey, E. M. Perry Point, Md., USA
Controlled studies of tranquilizing and anti-depressant drugs in 2,000 hospital patients.
Jus, A. Warsaw, Poland
Conditioned reflex experimental research in the evaluation of therapeutic mechanism of action of some psychotropic drugs.
Kalinowsky, L. B. New York, New York, USA
The place of the older and newer somatic treatment in psychiatry.

PSYCHOPATHOLOGY

Vinar, O. Prague, Czechoslovakia
Higher nervous activity in the schizophrenias and the LSD induced state.

PSYCHOPHARMACOLOGY

Akimoto, H. Tokyo, Japan (M. Nakakuki, Y. Honda, Y. Takahashi, Y. Machiyama)

Clinical evaluation of the effect of central stimulants, MAO-inhibitors and imipramine in the treatment of affective disorders.

Anastasopoulos, G. Thessaloniki, Greece (H. Photiades)
Research with LSD on relatives of schizophrenic patients.

Ayd, F. J. Baltimore, Md., USA
Long-term imipramine therapy: clinical and laboratory observations.

Bagdon, R. E. Nutley, N. J., USA (A. Boris)
Toxical investigations of Librium (R), a tranquilizing agent.

Bajusz, E. Montreal, Canada (H. Selye, R. Strebel)
Neuroleptic phenothiazine derivatives and stress-induced cardiac necroses.

Berthier, C. Bron, France
Les neuroplégiques sédatifs en thérapeutique psychiatrique.

Boittelle, G. Villejuif, France (C. Boittelle-Lentulo)
Un nouveau neuroleptique à action majeure, ses indications en psychiatrie, ses résultats: le Ro 4-0403.

Collard, J. Liége, Belgium
Intérét et analyse d'une nouville séries chimique de neuroleptiques: les butyrophenones.

Cossa, P. Nice, France (F. Paoli, G. Darcourt)
Sur l'utilisation de l'iminobenzyl (Tofranil) comme thérapeutique de la douleur.

Davies, B. M. Beckenham, England
Sernyl: its use in psychiatry.

Dimascio, A. Boston, Mass., USA (M. Rinkel, C. Atwell)
Personality and psychotomimetic drugs.

Feer, H. Basel, Switzerland
Vergleich der klinischen eigenschaften von drei phenothiazinähnlichen substanzen.

Barahona Fernandes, H. J. Lisbon, Portugal
Pharmacopsychotherapie.

Fontana, A. E. Olivos, Argentina
The clinical use of hallucinogenic drugs.

Fox, R. Kent, England
Safe sedation? A controlled trial of "megimated" amylobarbitone.

Gore, C. P. Yorkshire, England (James G. McComisky)
A study of the comparative effectiveness of Librium, amylobarbitone and a placebo in the treatment of tension and anxiety states.

Heise, G. A. Nutley, N. J., USA (H. McConnell)
Differences between chlordiazepoxide-type and chlorpromazine-type action on "trace" avoidance.

Henne, M. St. Venant, France (S. Henne, S. Kog, M. Tonnel)
Cinq années de recul dans l'emploi personnel de la levomepromazine (7044 RP, Nozinan) chez 500 hospitalisés.

Itil, T. Erlangen, Germany
Die veränderungen der pentothal-reaktion im EEG während der behandlung der psychosen mit psychotropen drogen.

Kinross-Wright, J. Houston, Texas, USA
Clinical pharmacology of some newer phenothiazine analogues.

Michaux, L. Paris, France (Perpiniotis)
Etude pharmacodynamique du G 22355.

Ostow, M. New York, New York, USA
Psychoanalysis and psychiatric drug therapy.

Quadbeck, G. Homburg, Germany
Der einfluss von psychopharmaka auf die blut-hirnschranke im tierversuch.

Rokhlin, L. L. USSR
Some principles of pharmacological therapy in the light of Pavlovian physiological teaching.

Vanni, F. Milan, Italy (F. Riggi)
The experimental evaluation of biological effects of "Tofranil" associated with monoamine oxidase inhibitors or neuroleptic drugs.

Werboff, J. Detroit, Mich., USA (J. S. Gottlieb, D. F. Caldwell)
Behavioral effects of prenatal administration of psychotropic drugs.

PSYCHOPHYSIOLOGY

Astrup, C. Oslo, Norway
Conditional reflex studies of schizophrenics treated with ataraxic drugs.

Burrell, C. New York, New York, USA (A. E. Burnett)
Blood cortisol levels and psychopathology.

Simpson, G. Orangeburg, New York, USA (N. S. Kline)
Histamine wheal formation and mental illness.

Timofeev, N. N. USSR (A. N. Timofeeva, E. A. Timofeeva, T. K. Kashkarova)
Clinical and psychopharmacological characteristics of hypopathy.

PSYCHOTHERAPY

Savage, C. Woodside, Calif., USA
The uses and abuses of LSD in psychotherapy.

SCHIZOPHRENIA

Bigelow, N. Marcy, New York, USA (A. Sainz, F. Ozerengin, J. Schneider)
Proketazine in the treatment of chronic schizophrenia.

Symposium on the Research into the Influence of Psychotropic Drugs on Higher Nervous Activity

Held in Prague, Czechoslovakia, October 31-November 3, 1961, this symposium was sponsored by the Society for the study of Higher Nervous Activity Czechoslovak Medical Society. Proceedings of the symposium will be published sometime in the future in *Activitas Nervosa Superior*. The papers delivered at the symposium, arranged according to subject matter, are listed below.

GENERAL METHODOLOGY

Techniques for maintaining behavior under conditions of intermittent reinforcement and their applications to the development of complex repertoires. C. B. Ferster (Indiana University Institute of Psychiatric Research, Indianapolis, USA).

Applied physiology of higher nervous activity in pharmacology and toxicology. M. Horváth, E. Frantík, and J. Formánek (Institute of Industrial Hygiene and Occupational Diseases, Dept. of Physiology of Higher Nervous Activity, Prague).

Conditioned reflex experiments in the evaluation of mechanism of therapeutic action of some psychotropic drugs. A. Jus (Warsaw Academy of Medicine, Psychiatric Clinic, Pruszkow k/ Warsaw).

Psychotropic drugs and autoencephalic stimulation. J. Olds (University of Michigan, Dept. of Psychology, Brain Research, Ann Arbor, Michigan, USA).

The possibilities of the use of conditioned reflex methods in psychiatric clinic. O. Vinar (Psychiatric Research Institute, Dept. of Psychopharmacology, Prague).

On pharmacological modification of higher nervous activity in experimental animals. Z. Votava, O. Benesová, J. Metysová, and M. Sousková (Research Institute of Pharmacy and Biochemistry, Dept. of Pharmacology, Prague).

EXPERIMENTAL PSYCHOPHARMACOLOGY IN ANIMALS

Differential psychopharmacological patterns of action in rats. K. Bättig (Dept. of Industrial Hygiene and Work Physiology, Swiss Federal Institute of Technology, Zurich).

The influence of septum cells on pharmacological reactions of the hippocampus in rabbits. F. von Brücke (Institute of Pharmacology, Medical Faculty, Vienna).

Electrophysiological comparison of the effects of tranquilizers in animals with implanted electrodes. O. Benesová, Z. Bohdanecky, and Z. Votava (Institute of Pharmacology, Medical Faculty of Hygiene, Research Institute for Pharmacy and Biochemistry, Prague).

The use of spreading depression in neurology. Z. Bohdanecky, J. Bures, O. Buresová, J. Necina, and T. Weiss (Institute of psychotropic drugs. J. Roubícek (Psychiatric Research Institute for Pharmacy and Biochemistry, Research Institute of Natural Substances, Prague).

New class of neuroleptics: N, N'-disubstituted piperazines. J. R. Boissier (Institute of Pharmacology, Faculty of Medicine, Paris).

(No title given). S. A. Corson, and E. O'Leary Corson (Research Division, Columbus Psychiatric Institute, Ohio State University Health Center, Columbus, Ohio, USA).

The influence of meprobamate on conditioned and unconditioned visceral and motor defense responses. R. A. Dykman, J. E. Peters, W. G. Reese, and L. D. Seager (University of Arkansas Medical Center, Little Rock, Arkansas, USA).

X-ray irradiation and psychotropic effects of chlorpromazine. A. Danysz, and T. Zaczek (Dept. of Pharmacology, Academy of Sciences, Bialystok, Poland).

High dietary tryptophan intake: methods used to investigate possible behaviour changes in the monkey. G. Ettlinger (Institute of Neurology, London W. C. 1).

The role of the systematic analysis of the environmental control of operant behavior in analyzing the effects of CNS drugs. C. B. Ferster (Indiana University of Medicine, U.S.A.).

Researches concerning psychopharmacology of noradrenaline. R. Floru, A. Costin, M. Sterescu-Volanschi, and V. Nestianu (Institute of Normal and Pathological Physiology "Prof. D. Danielopolu," R. P. R. Academy, Bucharest).

Research of the effect of psychopharmaca on the higher nervous activity by investigating the autonomic reactions of dogs to stimuli from the surroundings. K. Fronková, and V. Ehrlich (Institute for Diseases of Blood Circulation, Prague).

Analysis of parameters of the action of tranquilizing drugs on different conditioning test in the rat. G. L. Gatti, and D. Bovet (Research Institute of Medicine, Rome).

Some aspects of central action of adrenaline. V. Havlícek (Laboratory of Higher Nervous Activity, Faculty of Medicine, Olomouc, Czechoslovakia).

On the importance of the individual excitability of the central nervous system in the analysis of centrically acting pharmaca. K. Hecht (Institute for Cortico-Visceral Research, Berlin-Buch).

Comparative investigations of some centrically acting drugs by means of a conditioned-reflectory motorial feeding method and a conditioned-reflectory motorial escape method. K. Hecht (Institute for Cortico-Visceral Research, Berlin-Buch).

Some effects of morphine on adaptive and learning behavior. J. Jacob (Dept. of Pharmacology, Pasteur Institute, Paris).

Selective testing of antidepressive-agents. J. Knoll, and B. Knoll (Institute of Pharmacology, Faculty of Medicine, Budapest).

The influence of insulin on higher nervous activity. A. Kreindler, and J. Ungher (Pavlov's Neurological Institute, Academy of Sciences, Bucharest).

Some technical problems in evaluating the behavior effects of drugs. M. Sidman (Walter Reed Institute of Research, Washington, D. C., USA).

Evaluation of central pharmacological actions in animals with lesions of the basis cerebri. G. Tonnini, M. L. Riccioni, G. Missere, and M. Babbini (Institute of Pharmacology, Faculty of Medicine, Bologna).

Registration of the extinction of EEG component as a method for analysis of influence of drugs on higher nervous activity. V. Valdman (I. Pavlov Med. Institute, Dept. of Pharmacology, Leningrad).

On correlation between the antiserotonine and the depressor action of some phenothiazine derivatives. J. Venulet (Dept. of Pharmacology, Drug Research Institute, Warsaw).

The supramaximal electroshock seizures pattern and anticonvulsive effectiveness of phenobarbital and dilantine in the states of cholinergic stimulation in rats. B. Zablocka (Institute of Pharmacology, Faculty of Medicine, Warsaw).

CLINICAL PSYCHOPHARMACOLOGY

Salivation as an index of higher nervous activity: a physiological correlate of diagnosis and treatment outcome in depression. B. L. Busfield, and H. Wechsler (Massachusetts Mental Health Center, Boston, Mass., USA).

Multidimensional approach to psychopharmacological experiment. E. Kuhn, K. Rysánek, V. Vítek, M. Vojtechovsky, and S. Grof (Research Institute for Human Nutrition and Psychiatric Research Institute, Prague).

The measurement of autonomic activity in depressive patients during sodium amytal sedation. I. Martin (Institute of Psychiatry, Maudsley Hospital, London).

Contribution of electroencephalography to the evaluation of psychotropic drugs. J. Roubícek (Psychiatric Research Institute, Prague).

*Use of Chi-Square in Simple Crossover Designs**

In the clinical evaluation of new drugs, many investigators employ a simple crossover design, where half of the patients first receive the active drug for a given period of time, followed by a switch to an inert placebo for a similar period of time; the other half of the patients begin the trial on placebo, and are subsequently switched to the active drug. When the criterion measure consists of an overall rating of, say, "Improved" or "Non-improved," the data lend themselves to a chi-square analysis. It is clear, however, that in many clinical circles, this analytic technique is being consistently misused, despite the fact that the various pitfalls and underlying assumptions have been widely emphasized (Lewis and Burke, 1949). The purpose of this brief note is to demonstrate the proper use of chi-square, and also to show that, when incorrectly used, the clinical research investigator may increase the probability of prematurely discarding an effective drug.

Without going into the pros and cons of utilizing patients as their own controls in clinical psychopharmacology, assume that an investigator has tried out a new compound on 100 patients selected from some defined population. We are not concerned here with any of the methodological problems associated with executing the study; it is assumed that all necessary controls and precautions were utilized to prevent differential sources of error from biasing the resulting data. (It is further assumed that, for whatever reasons, the investigator was forced to use a dichotomous criterion measure, rather than a more desirable continuous measure.) At the conclusion of the study, the investigator tallies his results, and reports the data in Table 1.

TABLE 1. *Number of Patients Showing Improvement and Non-Improvement under Drug and Placebo Conditions (Total Number of Patients=100)*

	Improved	Non-Improved	Total
Drug	60	40	100
Placebo	50	50	100

Noting that 60 per cent of his patients improved when they were on the active drug, compared to only 50 per cent improvement when they were on placebo he now asks himself whether the difference is "statistically significant."

Since the investigator has a total of 200 different observations, his first inclination may be to carry out a conventional chi-square analysis, based upon the data of Table 1. With "o" representing observed frequencies, and "e" referring to expected frequencies:

	Imp	Non-Imp	
	A	B	
Drug	60	40	100=A+B
	C	D	
Placebo	50	50	100=C+D
	110	90	200=N
	A+C	B+D	

$$\chi^2=\Sigma\frac{(o-e)^2}{e}$$

$$=\frac{(60-55)^2}{55}+\frac{(40-45)^2}{45}+\frac{(50-55)^2}{55}+\frac{(50-45)^2}{45}$$

$$=2(.455)+2(.556)=2.02$$

Check:

$$\chi^2=\frac{N(AD-BC)^2}{(A+B)\ (C+D)\ (B+D)\ (A+C)}$$

$$=\frac{200}{99}=2.02$$

Aside from the fact that the resulting chi-square of 2.02 (for one degree of freedom) is nowhere near an acceptable significance level,[1] *this procedure is totally inappropriate* for two basic reasons.

The distribution of chi-square clearly assumes independence of observations, and since the patients are used as their own controls, there are only *100* independent observations. Caution: when the total number of observations exceed the total number of subjects used in the experiment, it is likely that the assumption of independence is being violated.

The second reason for the incorrectness of the above procedure is even more important: the fundamental hypothesis which the investigator wishes to test is *not* being tested! Actually, if the investigator should use the chi-square of Table 1 as evidence to accept the null hypothesis (i.e., that the drug and placebo are equally effective), he may or may not be making a correct decision, but one cannot tell without additional information.

* Prepared by Seymour Fisher, Ph.D., Chief, Special Studies Unit, Psychopharmacology Service Center.

[1] A correction for continuity always gives a more conservative estimate of the chi-square value, in this instance reducing the chi-square from 2.02 to 1.42. Parenthetically, the investigator might have "tested" his hypothesis by evaluating the difference between his observed percentages, 60 per cent Drug and 50 per cent Placebo, with each percentage based upon a denominator of 100. The standard formula for evaluating this difference (in the absence of any further information) assumes independent groups, and would lead to exactly the same probability value as the chi-square test (since, for one degree of freedom, $z^2=\chi^2$).

In order to appropriately test the hypothesis of a drug-placebo difference, it is necessary to know how each patient behaved on both drug and placebo. This information is not given in Table 1, but without it the investigator cannot hope to get an answer to his question. For illustrative purposes, Table 2 indicates the correct way in which the four-fold table should be set up. Knowing that 60 per cent of the patients improved on drug and 50 per cent improved on placebo, Tables 2A and 2B give hypothetical results consistent with this information.

It is instructive to examine the two sets of results and guess whether Table 2A or Table 2B offers the strongest evidence of the drug's effectiveness. In Table 2A, 50 of the 60 improved drug patients were non-improved on placebo; in Table 2B, only 12 of the 60 improved drug patients were non-improved on placebo. If "intuition" suggests that Table 2A gives better evidence for the drug-placebo difference, take a break and try again. The data in Table 2A yield (vide infra) a chi-square of 0.90 (nonsignificant), while the data in Table 2B give a chi-square of 5.79 (1 d.f., $p < .01$, one-tailed test).[2]

The crucial consideration in the appropriate analysis is that the investigator is *not* interested in those patients who respond the same to both placebo and drug administration; such patients only constitute "noise" in the system. Rather, he is particularly concerned with those patients who improve on one agent and do not improve on the other. Thus, in each table only two of the four cells become relevant to the hypothesis, and one now sees that in Table 2B, of 14 patients who responded differently to the two agents, the split in favor of the drug is 12/2; in Table 2A, the split of 90 patients is 50/40, much closer to chance expectation.[3]

It is interesting to observe that the cells which are essentially irrelevant to the analysis contain two kinds of patients: (*a*) those who showed no favorable response to either the drug or the placebo, and (*b*) those who responded favorably to both the drug and the placebo. Since any "placebo reactors" present in the sample would be expected to fall in the latter group, the correct application of chi-square in this situation takes into account their presence: by eliminating "placebo reactors" from the final analysis, a procedure advocated by Jellinek (1946) and Beecher et al. (1953), the investigator has a better chance of discriminating between the effectiveness of the two treatments.

The appropriate chi-square analysis for data obtained when two responses are measured on each subject has been worked out by McNemar (1955) and is discussed in Siegel (1956). In fact, Mosteller (1959) has specifically emphasized its application to cross-over designs in clinical psychoharmacology, but it appears that its use is still not widely understood.

The correct formula for chi-square (corrected for continuity) in this type of situation is:

$$\chi^2 = \frac{([B-C] - 1)^2}{B + C}$$

where B and C refer to the frequencies in the two cells

TABLE 2. Four-fold Tables of Frequencies for Drug and Placebo Responses from the Same Patients. (Total Number of Patients=100). In both tables, the margins indicate that 60 per cent improved while on drug, and 50 per cent improved while on placebo.

2A

Drug \ Placebo	Imp	Non-Imp	
Imp	A 10	B 50	60
Non-Imp	C 40	D 0	40
	50	50	100

2B

Drug \ Placebo	Imp	Non-Imp	
Imp	A 48	B 12	60
Non-Imp	C 2	D 38	40
	50	50	100

[2] It should be noted that combining the data in this fashion gives no information about the possibility of sequence effects or medication-sequence interaction (i.e., whether administering the drug prior to or following placebo is a factor in determining the results).

[3] The reader should not be lured into disregarding the important differentiation between *statistical* and *practical* significance. It would be possible to obtain the same "significant" chi-square value of Table 2B from an experiment which utilized a total of 2,000 patients, with only 14 patients manifesting a changed response from one treatment to the other:

Drug \ Placebo	Imp	Non-Imp	
Imp	998	12	1010
Non-Imp	2	998	990
	1000	1000	2000

One would still correctly conclude a drug effect probably exists, but the extent and meaning of the effect are, to say the least, questionable (50.5 per cent of the patients improved on drug, compared to 50.0 per cent improvement on placebo). Undoubtedly the skilled clinician would proceed to take a close look at the 12 patients who improved on drug but did not on placebo, since the data suggest that the real drug effect may be most applicable to a special population whose defining characteristics must now be identified.

which tally subjects who have changed from one response to the other:

For Table 2A:

$$\chi^2 = \frac{(50\text{-}40\text{-}1)^2}{50 + 40} = \frac{81}{90} = 0.90$$

For Table 2B:

$$\chi^2 = \frac{(12\text{-}2\text{-}1)^2}{12 + 2} = \frac{81}{14} = 5.79$$

For a discussion of the limitations and assumptions involved in using this formula, see Siegel (1956). A general rule of thumb to keep in mind is that the preferred minimum total of patients in cells B and C should be 10 for a valid test of significance. When $B + C < 10$, an exact test can be made using the binomal rather than the chi-square distribution (Siegel, 1956).

SUMMARY

By taking into account the correlation within patients who are exposed to two different treatments in a crossover design, one has a much better chance of detecting valid treatment differences than if this correlation is ignored. Given categorical measures of "improvement" and "non-improvement," chi-square analysis is appropriate, but must be used in a modified form, based on independent observations. When the data are presented merely in terms of the percentage of patients who improved on drug compared to the percentage of the same patients who improved on placebo, one may not be able to say anything about the effectiveness of the drug without additional information concerning how each patient responded to both treatments. Using hypothetical examples, it is shown how a relatively unimpressive overall difference in percentage improvement may obscure a marked drug-placebo difference which can be detected by appropriate statistical analysis. If an investigator insists upon using the kind of crossover design under discussion (and there are many excellent reasons why this design should generally be avoided), it is particularly important for him to have a clear understanding of the processes and assumptions underlying his techniques of analysis.

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*New Drugs and Developments in Therapeutics**

A COUNCIL ON DRUGS' DIGEST

Drugs for Mental Illness

In 1954, there was introduced into American medicine, against a background of extensive European investigation, a drug with a remarkable diversity of effects: antihistaminic, antiemetic, sedative, as well as less prominent local anesthetic, hypothermic, adrenolytic, anticholinergic, and curare-like actions. The drug was, of course, chlorpromazine (Thorazine), a derivative of phenothiazine. Although it was by no means the first phenothiazine compound introduced, none of the earlier compounds met with the commercial success of chlorpromazine, which, in addition to its other uses, rapidly gained acceptance as a valuable agent for the alleviation of anxiety associated with mental illness. Doubtlessly prompted by this success, American pharmaceutical manufacturers subsequently introduced, and continue to introduce, a procession of other phenothiazine derivatives; more than a dozen are now available. Some are promoted primarily as "tranquilizers," others as antiemetic or antihistaminic agents. Many offer only questionable advantages over chlorpromazine, and some authorities doubt that any of these phenothiazine derivatives is clearly superior to chlorpromazine in the management of major psychoses.

Coinciding with the introduction of these compounds and of drugs of other chemical categories, there has been a resurgence of interest in the chemical or medicinal treatment of mental illness; the use of the

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so-called tranquilizer has increased proportionately. According to the results of one survey (American Druggist, Feb. 6, 1961), the proportion of prescriptions falling into the "sedative-tranquilizer" group recently reached an all-time high of 19.4 per cent. "Tranquilizers" (excluding barbiturates) constituted the major ingredient of 13 per cent of the prescriptions surveyed and in this respect were second only to antibiotics.

SIDE EFFECTS OF PHENOTHIAZINE COMPOUNDS

If phenothiazines are assumed to account for more than a small portion of the "tranquilizers" prescribed, these statistics must be viewed with some alarm, since, of the dozen phenothiazine compounds that have been reviewed by the Council for use in the treatment of neuropsychiatric disorders, none is free from potential serious toxicity; each has its characteristic array of side effects. The most serious complications include jaundice (usually reversible), blood dyscrasias, convulsive seizures, hypotension, disturbances of temperature regulation, excessive sedation, and atropine-like effects; these are described in the Council's statement on chlorpromazine (*New and Nonofficial Drugs*, 1961, p. 429).

Two additional groups of reactions seem to merit more detailed description here, since they are particularly troublesome with some of the newer phenothiazines: *extrapyramidal tract dysfunction* and *pigmentary retinopathy*.

Recognition of the fact that *extrapyramidal tract reactions* may be produced by the phenothiazines is very important in order to avoid an erroneous diagnosis of tetany, tetanus, encephalitis, meningitis, or other neurological disease. These symptoms seem to be especially common with piperazine-substituted compounds, which include fluphenazine (Permitril, Prolixin), perphenazine (Trilafon), prochlorperazine (Compazine), thiopropazate (Dartal), and trifluoperazine (Stelazine). The frequency of occurrence and the severity of the symptoms appear to be related to the amount of the drug administered; the symptoms can usually be controlled by reducing the dose or by administration of an antiparkinsonism agent. As far as is known, the symptoms are reversible and disappear when administration of the drug is discontinued. The reactions have been divided into the following three groups: (1) a parkinsonism-like syndrome with abnormal, shuffling gait, mask-like facies, postural abnormalities, rigidity, tremors, excessive salivation, and pill-rolling movements of the hand; (2) dyskinetic reactions, most commonly involving the muscles about the face and neck, and including perioral spasms, often with protrusion of the tongue, mandibular tics, difficulty in speech and swallowing, oculogyric crises, torticollis, hyperextension of the neck and trunk, and tonic and clonic contractions of other muscle groups; profuse sweating, pallor, and fever may accompany these symptoms, but consciousness is not lost and the patient remains fully alert; and (3) akathisia or motor restlessness, varying in intensity from feeling of inner disquiet to inability to sit still or sleep, complete intolerance of inactivity, and an appearance of continuous agitation.

Pigmentary retinopathy is discussed in the following comment on thioridazine, the only commercially available phenothiazine compound with which this complication has been associated.

NEWER PHENOTHIAZINE COMPOUNDS

Three newer phenothiazine compounds recently reviewed by the Council are fluphenazine hydrochloride (Permitil, Proloxin), trifluoperazine hydrochloride (Stelazine Dihydrochloride), and thioridazine hydrochloride (Mellaril). The usefulness of these drugs in the management of neuropsychiatric disorders is similar to that of chlorpromazine. There is as yet no convincing evidence that they have greater efficacy than the older phenothiazine compounds; the question of relative toxicity can be settled only after much more experience has been acquired; at the present time, therefore, there would seem to be no reason to choose these newer drugs in preference to phenothiazine compounds that have had more extensive usage and whose toxic and therapeutic potentialities have been more thoroughly explored.

The most troublesome *side effects* of *trifluoperazine* and *fluphenazine* are the manifestations of extrapyramidal neurological disturbances. The incidence of these effects is closely related to dosage. For example, they may be observed in more than 50 per cent of patients receiving large doses of trifluoperazine, whereas the incidence is only about 2 per cent when the dose is in the range of 2 to 4 mg. per day.

Administration of very large doses of *thioridazine* has been found to produce *pigmentary retinopathy*. This reaction is characterized by diminution of visual acuity, brownish coloring of vision, and impairment of night vision; examination of the fundus discloses deposits of pigment and, several weeks later, edema. To date, this syndrome has been observed only in patients receiving 1.6 gm. or more of thioridazine per day. Reversible impairment of vision, without detectable retinal change, is reported to have occurred in a patient in whom the daily dose was only 400 mg., but, one month earlier, this patient had received 1.2 gm. per day for a period of 12 days.

Jaundice has not yet been reported in patients

given thioridazine, and extrapyramidal disturbances are rare.

PHENOTHIAZINE COMPOUNDS IN THE MANAGEMENT OF NEUROSES AND MINOR EMOTIONAL DISORDERS

The Council has also recently considered the use of trifluprom azine hydrochloride (Vesprin) (*New and Nonofficial Drugs*, 1961, p. 447) in the treatment of neuroses and minor emotional and behavioral disorders and has concluded that its usefulness in the treatment of these conditions is comparable to that of other phenothiazine drugs. It is the opinion of the Council, however, that the phenothiazine derivatives, as a class, are less consistently effective in these disorders than in the major psychoses and that less toxic drugs, e.g., barbiturates, are available for the treatment of simple neuroses. It is emphasized that suppression of symptoms by the long-term administration of a drug, in lieu of accurate diagnosis and correction of underlying causes, constitutes poor medical practice, especially when the drug has the potential toxicity of the phenothiazine compounds. Because of the possible occurrence of serious reactions, the unsupervised use of phenothiazine compounds in patients who cannot be carefully observed by the physician must be avoided. Furthermore, it must be remembered that side effects which might not be serious in hospitalized patients may be exceedingly dangerous in outpatients. For example, drowsiness or lethargy, even though moderate, may not only decrease the patient's working efficiency, but may, in many situations such as driving an automobile or operating other vehicles or machinery, constitute a life-endangering hazard.

Publications and Other Information Materials

Listed below are a few publications and other informational materials, some of which may be obtained by writing to the Scientific Information Unit of PSC; the others, from the sources indicated. No attempt has been made to list all new publications in the *Bulletin*, but only those, e.g., Government reports and foreign publications, that might not ordinarily come to the attention of psychopharmacologists. Please write to the *Bulletin* if you know of any publications that you think should be listed.

MATERIALS AVAILABLE FROM THE PSYCHOPHARMACOLOGY SERVICE CENTER

Psychotropic Drug Use in a General Medical Outpatient Population. (Mimeographed)
Based on an analysis of data sampled from the Group Health Association in Washington, D. C., this study presents information on the use of drugs by a group medical practice in a single metropolitan area. It discusses the relationships between prescribing psychotropic drugs and the characteristics of the patients (e.g., age, sex, race, symptoms). Summaries of this study (and of the report cited next) are available in limited numbers for distribution. Requests should be sent to Seymour Fisher, Ph.D., Chief, Special Studies Unit, Psychopharmacology Service Center, National Institute of Mental Health, Bethesda 14, Maryland.

A Report on the Use of Psychotropic Drugs by Physicians in Private Practice in the United States, July 1959-June 1960. (Mimeographed)
This report, along with the one cited above, results from an attempt to identify factors associated with the decision to prescribe psychotropic drugs outside of the typical hospital setting. It discusses the variables which describe the physicians (e.g., age, experience, specialty) and particular drug classes (including the route of administration). See preceding item above for availability.

Psychopharmacology Service Center of the National Institute of Mental Health. December, 1960.
This little pamphlet describes the activities of the PSC, the research program and how grants are awarded, and the services which the Center offers to investigators working in this field. A copy has already been mailed to all recipients of the *Bulletin*. The Center has additional copies and will be glad to send them on request.

Psychopharmaca, A Bibliography of Psychopharmacology, 1952-1957. Compiled by Anne E. Caldwell. Washington, D. C. United States Government Printing Office, 1958.
This bibliography was out of print for several months. It has now been reprinted and the Center has copies for distribution.

Metabolic Studies and Analytical Methods for Phenothiazine Derivatives Used in Psychopharmacology: A selected annotated reference list (Mimeographed)
This reference list is made up primarily of articles concerned with analytical methods for the detection of phenothiazine derivatives used in psychopharmacology, together with a few general articles on the metabolism of these agents. The annotations are factual summaries; they are not evaluative or critical. The list is arranged alphabetically by author. Although a

few early articles are included, a more complete coverage is given to the period from 1954 to June 1961. The list is not an exhaustive, definitive bibliography of this topic.

Placebos in Psychopharmacology. (Mimeographed). This unannotated list contains 177 references to articles on the use and effect of placebos in psychopharmacology. It includes articles on such subjects as reactions to placebos, ethics of using placebos, and problems of placebos in research. The list is arranged in alphabetical order, by author, and grouped according to year of publication: prior to 1959, 1959, 1960, and 1961.

OTHER PUBLICATIONS

Proceedings of the International Symposium on the Extrapyramidal System and Neuroleptics, ed. by Jean-Marc Bordeleau, M. D. $12.00

Copies may be obtained by writing to Docteur J. M. Bordeleau, Institute Albert-Prévost, 6555 ouest, boulevard Gouin, Montreal 9, Canada.

More than 50 papers are contained in these proceedings; most are in English, the others in French; almost all are followed by English or French summaries. The subjects covered are: anatomy and physiopathology, pharmacology, Parkinson's disease, neurosurgical panel on neural mechanisms in extrapyramidal diseases and their treatment, extrapyramidal syndromes and clinical psychiatry, panel on clinical activity of neuroleptics, extrapyramidal reactions and dynamic theories, and a panel on the psychodynamic aspects of extrapyramidal reactions.

Patologia Mentale e Farmacologie [Mental disease and Pharmacology], by Antonio Balestrieri. Padova: Cedam, 1961. Bibliography, 258 pp., English summaries of each chapter. Price: L. 3500.

Action for Mental Health: the Final Report of the Joint Commission on Mental Illness and Health, 1961, New York, Basic Books, Inc., $6.75.

Empowered by the U. S. Congress to conduct a study of the mental health of the United States, the Joint Commission on Mental Illness and Health sets forth its findings and recommendations in this volume. Among its recommendations are: large increases in spending by Federal, State, and local governments for public mental health services; and the creation of totally new methods of mental health care.

Activitas Nervosa Superior is a new interdisciplinary journal carrying articles on psychopharmacology and related subjects. Published in Prague, Czechoslovakia, and edited under the auspices of the Society for the Study of Higher Nervous Activity, the journal publishes papers in Congress languages and in Czech, with Russian and English abstracts. The journal is published quarterly and may be obtained by writing to: ARTIA, Smecky 30, Praha 2, Czechoslovakia. The subscription is $7.40 per year.

Journal of Neuropharmacology will commence trimonthly publication early in 1962, the Pergamon Press, Ltd., hopes. The new journal will devote itself to experimental and clinical information relating to the substances and drugs acting on the central and peripheral nervous system. In addition to original papers in the field, the journal will publish analytic bibliographies. It will not publish reviews or monographs on neuropharmacology; a yearbook, *Progress in Neuropharmacology,* will be founded concurrently for that purpose. Manuscripts, in duplicate, should be sent, for the U.S.A., to B. B. Brodie, National Heart Institute, Bethesda 14, Maryland; for other countries, to C. Radouco-Thomas, Hôpital Cantonal, Geneva, Switzerland. Business communications (regarding advertising, subscriptions, reprints, etc.) should be addressed to Pergamon Press, Ltd., Headington Hill Hall, Oxford, England.

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Announcement

The third biennial meeting of the Collegium Internationale Neuro-Psycho-Pharmacologicum will be held September 2-5, 1962, in Munich, Germany. Specific topics will be covered each day in formal morning sessions, with afternoons reserved for individual papers on the same subject. While the papers for the morning sessions have already been selected, brief papers for the afternoon sessions may still be submitted by members and nonmembers. Scientists wishing to present papers at the afternoon sessions should submit titles and 300-word abstracts before February 1, 1962, to any of the appropriate members of the program committee:

Dr. B. P. Bradley
The Medical School
Hospital Centre
Birmingham 15, England

Dr. J. Thuillier
28, Rue Chardon-Lagache
Paris 16e
France

Dr. Hanns Hippius
Forschungabt. der Nervenklinik der Freien Universität
30, Ulmenallee
Berlin-Charlottenburg
Deutschland

Dr. Jonathan O. Cole
Psychopharmacology Service Center
National Institute of Mental Health
Bethesda, Md.
U.S.A.

Topics for the sessions, from Sunday through Wednesday, September 2-5, will be, respectively: The Methods of Measurement of Behavioral Changes with Drugs in Animals and in Man; The Effectiveness of Drugs in Relationship to Psychological and Social Forms of Treatment; Ten Years of Modern Psychopharmacology: Critical Assessment of Past, Present, and Future, (a) Basic Sciences, (b) Clinical Sciences; and Communications on Clinical and Experimental Studies with New Drugs.

Other questions concerning the Collegium should be addressed to either of the two secretaries of the CINP:

Prof. W. de Boor
Kerpener Str. 4
Koeln, Deutschland

Dr. Fritz A. Freyhan
Saint Elizabeths Hospital
Washington, D.C., U.S.A.

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